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SIPHONAPTERA: THE GENUS *OROPSYLLA* IN NORTH AMERICA*

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Fleas of the genus *Oropsylla* are characteristic ectoparasites of several species of fossorial rodents in the genera *Citellus* and *Marmota* of the family SCIURIDAE in North America and Asia. As *Oropsylla rupestris* (Jordan) and, to a lesser extent, *O. idahoensis* (Baker) have been found by Eskey and Haas (1940) to be experimental vectors of plague, and as their natural hosts have been found infected with plague in nature in many Western States and Canada, a taxonomic review of the genus *Oropsylla* seems appropriate.

Oropsylla Wagner and Ioff 1926

Male and Female: Typical ceratophylline fleas of medium to large size and of golden brown color. Eye prominent, gena rounded, not acute, frontal tubercle small (very small in *O. alaskensis*), maxillae long and acute, labial palpi large and long extending beyond the trochanters. A few small bristles present on the outside of the forefemur, inside of hind coxae with very numerous long fine bristles near anterior margin from base to apex. Five pairs lateral plantar bristles on the fifth segment of each tarsus though odd specimens may have 6 in some rows, proximal pair of plantar bristles not displaced inward. Well developed pronotal ctenidium, apical spinelets on anterior abdominal tergites and sometimes on metanotum. Basal abdominal sternite with ventral bristles but lacking a patch of lateral bristles except in ♀ of *O. alaskensis* which has 4 or 5. Some abdominal tergites with 3 definite rows of bristles and additional scattered bristles. Lower bristles on most abdominal tergites below the level of the row of spiracles.

Female: Sternite VII entire without prominent lobes or incision, head of spermatheca ovate, slightly longer than broad, tail about as long as head and may be expanded in *O. arctomys* and *O. rupestris*, and in all species has prominent terminal appendage (a most typical character of the genus). Three and often 4 large antepygidial bristles on each side, stylet with 2 to 4 lateral and a long terminal bristle.

Male: Sternite VIII long and narrow with a terminal or subterminal group of 4 to 6 long bristles but without a membranous appendage. One large, one minute antepygidial bristle. Process and finger of the clasper broad with numerous bristles but no black spines. Levers of penis with about one complete convolution.

The original description of the genus by Wagner and Ioff (1926) is quoted:

Der massive Rüssel ist stark verlängert; das ganze letzte Gliedchen der Unterlippentaster überragt den Trochanter; dies Gliedchen ist nicht weniger als doppeltlang im Vergleich zu dem vorletzten Gliedchen und länger als das 3. und 4. zusammen. Die frontale Reihe ist nicht vollkommen entwickelt, es sind davon bloß die Eckborsten erhalten (gewöhnlich 2) so wie die oberen—bis auf 3 Härchen—recht schwache und weit gegen den Rücken fortgeschobene. Die Scheitelborstenreihen—rudimentär. Die Antennenkeule ist bei dem ♀ von einer etwas verlängerten ovalen Form, bei dem ♂ aber ist sie stark verlängert. Die Anzahl der Antepygidialborsten ist beim ♂—1, bei der ♀—3. Das achte Abdominalsternit der ♀ ist halbrudimentär, kurz, mit langen Borsten. Receptaculum seminis—mit einem Chitinauswuchs, am Ende seiner engen Distalabteilung, sein Reservoir (Proximalabteilung) von ovaler Form.

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* Contribution from Rocky Mountain Laboratory, (Hamilton, Montana), Division of Infectious Diseases, National Institute of Health.

Collection data of *Oropsylla arctomys* (Baker)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18079	Alaska	Fairbanks	7-17-1937	<i>M. monax ochracea</i>	4♂, 6♀	C. B. Philip
	British Columbia	Eagle River (North Fork)	5-22-1938	<i>M. caltigata okanagana</i>	1♂	G. P. Holland
	"	Quick	6-5-1943	<i>Canis latrans</i> (coyote)	2♂, 2♀	"
	"	Wigwam Mine	5-21-1939	<i>M. monax petrensis</i>	2♂, 1♀	"
	Saskatchewan	Varenby	8-17-1931		2♀	"
	"	Big River	8-15-1942	<i>Marmota</i> sp.	1♂, 3♀	"
	"	Kendall	9-18-1943	Man	2♀	"
6035	Canada	Ontario	10-1-1930	<i>Marmota</i> sp.	1♂	U.S. Nat. Museum
14537	Montana	Ravalli Co.	4-25-1930	<i>M. flaviventris</i>	1♂	Rocky Mountain Laboratory
15828	Wyoming	Albany Co.	6-15-1938	"	1♀	"
15836	Minnesota	Cloquet	4-9-1936	<i>M. monax</i>	1♂	U.S. Nat. Museum
15877	Michigan	Berrien Co.	7-11-1919	"	1♀	Univ. Michigan
15945	"	Cheboygan Co.	7-29-1919	"	2♂, 1♀	G. W. Luttermoser
15878	"	Gogebic Co.	July 1935	"	1♂, 2♀	Univ. Michigan
15881	"	"	Aug. 1920	"	1♂, 1♀	"
15883	"	"	6-30-1920	"	3♂, 2♀	"
18250	"	"	Aug. 1920	"	1♂, 1♀	"
16503	"	"	Oct. 1920	<i>Mephitis</i> sp.	4♂, 6♀	"
	Lincoln, Penobscot Co.		8-11-1920	"	1♂, 1♀	"
	Horseshoe Island		Oct. 1940	"	1♂, 1♀	"
	Keeseville, Essex Co.		6-4-1932	<i>M. monax</i>	1♂, 1♀	W. Clayton through Robt. Traub
	North Elba		5-29-1918	"	3♂, 3♀	L. C. Stegeman
	Peterboro		7-17-1926	"	1♂, 1♀	U.S. Nat. Museum
	Rockland Co.		7-9-1900	"	1♀	"
	Syracuse, Onodago Co.			"	1♂, 1♀	(types)
	Monroe			"	6♂, 9♀	"
	Abington			"	1♂, 1♀	L. C. Stegeman
	Belmont			"	2♂, 2♀	U.S. Nat. Museum
	Charles River Village			"	1♂, 1♀	"
	Holbrook		4-12-1924	<i>Mephitis</i> sp.	1♂	"
	Needham, Norfolk Co.		7-8-1926	<i>M. monax</i>	1♂, 1♀	"
	Newton Center		6-13-1938	<i>Mephitis</i> sp.	1♂	"
	Sagamore		11-11-1923	<i>M. monax</i>	1♂	"
	Canobie Lake		5-13-1924	"	1♂, 1♀	"
	Concord		3-26-1927	"	1♂, 1♀	"
	"		9-20-1926	"	3♂, 1♀	"
	"		7-29-1934	"	1♂, 1♀	"
	"		July 1892	<i>Mustela</i> (mink)	1♂, 1♀	"
	"		Mar. 1901	<i>Vulpes</i> (red fox)	1♂, 1♀	"
	"		5-15-1926	"	1♂, 1♀	"
17403	New Hampshire					
16236	"					

Collection data of *Oropsylla rupestris* (Jordan)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
8821	Alberta	Calgary	6-28-1940	<i>C. richardsoni</i>	2♂, 2♀	G. P. Holland (topotypes)
	"	Edmonton	8-28-1932	<i>Citellus</i> sp.	1♂, 6♀	Rocky Mountain Laboratory
	Saskatchewan	High River	7-28-1938	<i>C. richardsoni</i>	1♀	G. P. Holland
	"	Estevan	8-6-1942	<i>Mustela</i> sp.	1♀	"
	"	Rock Glen	9-9-1942	<i>C. richardsoni</i>	1♂, 1♀	"
	"	Saskatoon	6-6-1941	"	2♂	"
18777	"	"	6-6-1941	"	1♂, 3♀	L. G. Saunders
	"	"	10-10-1940	<i>Mustela</i> sp.	1♀	G. P. Holland
18105	"	Val Marie	7-8-1942	"	1♂, 1♀	Rocky Mountain Laboratory
18199	Montana	Blaine Co.	5-25-1937	<i>C. richardsoni</i>	1♂, 1♀	Montana State College
10054	"	Gallatin Co.	3-11-1929	"	1♂, 3♀	Rocky Mountain Laboratory
	"	"	3-15-1934	"	1♀	"
	"	"	4-7-1940	"	1♂, 15♀	"
	"	"	March 1940	"	14♂, 7♀	"
	"	Glacier Co.	6-14-1940	"	1♂, 1♀	"
	"	Wheatland Co.	8-27-1935	"	8♂, 9♀	"
18967	N. Dakota	Barnes Co.	8-10-1941	"	2♂, 4♀	E. C. Cates, Montana State College
19099	"	Benson Co.	8-28-1941	"	3♂, 1♀	Rocky Mountain Laboratory
18992	"	McHenry Co.	8-20-1941	"	7♂, 6♀	"
19088	"	"	8-25-1941	<i>Citellus</i> sp.	5♂, 3♀	"
19117	"	Pierce Co.	9-2-1941	<i>C. richardsoni</i>		"

Collection data of *Oropsylla idahoensis* (Baker)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18241	Alaska	Cantwell	7-10-1939	<i>Citellus</i> sp. (several hosts)	9♂, 17♀	C. B. Philip
17280	"	Little Koninski Island and Shumagin Island	4-14-1940	"	1♂, 3♀	J. Warwick
18239	"	Rapids	July 1980	" = " <i>Spermophilus</i> "	3♂, 2♀	U.S. Nat. Museum by F. H. Beun
17281	British Columbia	Richardson Hiway	7-13-1939	" (several hosts)	2♂, 20♀	C. B. Philip
	"	Isaac Creek	9-13-1941	"	2♂, 4♀	J. Warwick
	Canada	Mt. Begbie	8-8-1941	<i>C. columbianus</i>	2♂, 2♀	G. P. Holland
	Alberta	Jasper National Park	7-2-1940	"	1♂, 1♀	"
	"	Banff	6-3-1943	"	1♂, 1♀	"
	Washington	Cofax, Whitman Co.	7-2-1939	<i>C. lateralis</i>	2♂, 5♂, 2♀	"
8220A	"	Iron Springs	4-21-1927	<i>C. columbianus</i>	1♀	U.S. Nat. Museum
8250A	Oregon	Harney Co.	5-23-1930	<i>Thomomys fuscus</i>	1♂	"
	"	"	7-17-1932	<i>C. columbianus</i>	1♂	Rocky Mountain Laboratory
	"	"	7-18-1932	<i>C. lateralis</i>	2♂, 2♀	"
9180B	"	Keno, Klamath Co.	8-10-1934	<i>C. columbianus</i>	1♂, 7♀	U.S. Nat. Museum
13177	"	Lake Co.	5-20-1933	<i>C. collinus</i> sp. (2 hosts)	1♂, 4♀	Rocky Mountain Laboratory
13417	Idaho	Arco, Butte Co.	5-8-1937	<i>C. armatus</i>	1♂, 5♀	"
12707	"	Moscow Mrs. Latah Co.	6-8-1936	<i>C. columbianus</i>	5♂, 5♀	" (topotypes)
13244	Montana	Beaverhead Co.	7-28-1936	<i>C. lateralis</i>	2♂, 1♀	"
13246	"	"	6-21-1937	<i>Asio velsonianus</i> , owl	1♂	"
	"	"	June & July 1937	<i>C. armatus</i> (several hosts)	3♀	"
13358	"	"	7-19-1937	<i>Pica pica hudsonia</i> , magpie	1♂	"
14128	"	"	July 1938	<i>C. columbianus</i> (several hosts)	8♂	"
14182	"	"	July 1938	<i>Citellus</i> sp., probably <i>r. elegans</i>	2♂, 10♀	"
	"	"	(4 other collections this host and county.)	Nest of <i>Buteo</i> , hawk	1♂, 1♀	"
18698	"	"	6-27-1936	"	2♀	"
	"	Flathead Co.	7-6-1936	<i>C. columbianus</i>	1♂, 4♀	C. P. Haight, Montana State College
	"	Granite Co.	5-1-1941	"	1♀	"
	"	Lake Co.	5-17-1940	"	3♂, 4♀	Rocky Mountain Laboratory
	"	Madison Co.	6-11-1940	"	1♂	"
12671	"	"	6-15-1940	<i>C. armatus</i>	5♂, 7♀	"
12672	"	"	June 1936	<i>Citellus</i> sp. (5 hosts)	1♀	C. P. Haight, Montana State College
12669	"	"	7-24-1940	<i>C. armatus</i>	3♂, 9♀	Rocky Mountain Laboratory
2394	"	Missoula Co.	May 1940	<i>C. columbianus</i>	1♂, 4♀	"
14076	"	Ravalli Co.	4-17-1923	<i>C. lateralis</i>	3♂, 10♀	"
	"	"	(Numerous other collections from same host and locality.)	<i>C. columbianus</i> (3 hosts)	1♀	C. P. Haight
	"	"	July 1938	(Numerous other collections from same host and locality.)	10♂, 5♀	Rocky Mountain Laboratory
14414	Wyoming	Silver Bow Co.	7-16-1940	<i>C. r. elegans</i>	1♂, 1♀	"
14449	"	Albany Co.	5-14-1938	<i>C. lateralis</i>	3♂, 3♀	"
14450	"	"	5-24-1938	" (3 hosts)	2♀	"
14417	"	"	May 1938	"	1♂	U.S. Nat. Museum
	"	Natrona Co.	5-14-1938	"	1♂, 2♀	"
	"	Yellowstone Park	8-17-1923	<i>Citellus</i> sp.	1♂, 2♀	"
	"	Salina	7-26-1923	<i>C. lateralis</i>	1♂, 1♀	"
	Utah	"	8-2-1923	<i>C. armatus</i>	2♂, 1♀	"
	"	Strawberry Valley	6-1-1942	"		"

Collection data of *Oropsylla idahoensis* (Baker)—Continued.

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18117 18951 16844 and other numbers	California Colorado	San Bernardino Co. Archuleta Co. Boulder Co.	5-4-1936 6-11-1932 April, 1 May, June, July, 1940 8-11-1931	<i>C. lateralis</i> " " (many hosts)	1 ♀ 3♂, 1 ♀ 70♂, 76♀	Rocky Mountain Laboratory " "
8008A 15692 15665 and other numbers	"	Conejos Co.	(Numerous specimens same locality and host.)	"	1♂, 1 ♀	Montana State College
15544 15314 15710 15703 and other numbers	"	Gilpin Co. Lake Co.	7-11-1932 6-26-1939 6-22-1939	<i>Cynomys</i> sp. <i>C. lateralis</i> <i>Citellus</i> sp. (5 hosts)	2 ♀ 3♂, 3 ♀ 4♂, 7 ♀	Rocky Mountain Laboratory " "
13729 15663 and other numbers	"	Larimer Co. Moffat Co. Park Co.	2-23-1939 6-6-1938 6-30-1939 6-30-1939	" <i>C. r. elegans</i> <i>Citellus</i> sp. <i>Cynomys</i> sp. (4 hosts)	2♂ 1♂ 1♂, 2 ♀ 16♂, 32♀	" " " "
16925 11000D and other numbers	"	Pike Co. Summit Co.	7-25-1937 6-22-1939	<i>C. lateralis</i> <i>Citellus</i> sp. (4 hosts)	5♂, 1 ♀ 6♂, 8 ♀	" "
	Arizona	Tellur Co. Coconino Co.	8-6-1924 5-21-1935	<i>C. lateralis</i> (2 hosts) " (3 hosts)	2♂, 1 ♀ 2♂, 10 ♀	" "

Wagner and Ioff (1926) described the genus *Oropsylla* in Russian, and also in German as quoted above, naming as the genotype *Ceratophyllus silantiewi* Wagner 1898, a parasite of Asiatic marmots, *Marmota bobac* and *M. siberica*. They also included in the genus a new species, *O. ilovaiskii*, a species described by Jordan and Rothschild (1911) as *C. crassus* from *Marmota robusta* in China and "several others." Wagner (1930) considered *O. crassus* as a subspecies of *O. silantiewi* and added *C. elana* Jordan 1929 to the genus. He also tentatively referred *C. mandarinus* to *Oropsylla*.

In a discussion of the American species of fleas on ground squirrels and marmots, Wagner (1929) referred six species to the genus *Oropsylla*, namely, *C. alaskensis* Baker 1904, *C. bruneri* Baker 1895, *C. idahoensis* Baker 1904, *C. montanus* (Baker) 1895, *C. rupestris* Jordan 1925, and *C. tuberculatus* Baker 1904. Jordan (1933) reviewed the American species of the genus *Ceratophyllus* (*sensu lato*) and recognized or described a total of 18 genera. In this review, the genus *Oropsylla* was redefined and the American species were restricted to *O. alaskensis*, *O. arctomys*, *O. idahoensis* and *O. rupestris*. He also assigned the Asiatic species *mandarinus*, which Wagner had tentatively referred to *Oropsylla*, to the new genus *Diamanus*. Ewing and Fox (1943), in a treatise on the North American fleas, placed *Diamanus* Jordan and *Opisocrostitis* Jordan as subgenera of *Oropsylla*, an arrangement with which the writer does not agree.

Oropsylla arctomys (Baker) 1904 = *Aetheopsylla septentrionalis* Stewart and Holland 1940

1929. *Oropsylla arctomys* (Baker). Jordan, Nov. Zool. 35: 32.
 1936. *Oropsylla arctomys* (Baker). Ioff, Zeit. Parasit. 9: 73-124.
 1939. *Oropsylla arctomys* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178.
 (Bibliography of species up to 1939.)
 1939. *Oropsylla arctomys* (Baker). Jellison and Kohls, Pub. Health Rept. 54: 2022.
 1940. *Oropsylla arctomys* (Baker). Eskey and Haas, Pub. Health Rept. 254: 73.
 1940. *Oropsylla arctomys* (Baker). Fox, Fleas of Eastern United States, pp. 45-46.
 1940. *Aetheopsylla septentrionalis* Stewart and Holland, Can. Ent. 72: 41-42.
 1942. *Oropsylla arctomys* (Baker). Fuller, Ent. News 53: 137.
 1943. *Oropsylla* (*Oropsylla*) *arctomys* (Baker). Ewing and Fox, U. S. Dept. Agric. Misc. Pub. No. 500: 49.
 1943. *Oropsylla arctomys* (Baker). Fuller, Jour. New York Ent. Soc. 51: 5.
 1943. *Oropsylla arctomys* (Baker). Fuller, Bull. Brook. Ent. Soc. 38: 20.
 1943. *Oropsylla arctomys* ———. Bell and Chalgren, Jour. Wildlife Management 7: 275.

Male: Length 2.9 mm,¹ frontal tubercle prominent in some specimens, much reduced in others, labial palps equaling forefemur, preoral bristles 1 or 2 pairs, 1 large and numerous small bristles along dorsal border of antennal groove; pronotal ctenidium of 16 to 18 spines in specimens from eastern United States, and 20 to 22 spines in Alaskan specimens; apical spinelets on each side as follows: metanotum 1, abdominal tergites I to III, 2-1-1 respectively; 1 large and 1 very small antepygial bristle on each side. Only one acetabular bristle on each side. This character was mentioned in the original description. It is found to hold in the long series available for study and in most specimens of the closely related species, *O. rupestris*. Moveable process of the clasper broadly club-shaped, convex on anterior margin; sternite VIII with 4 large terminal and 3 or 4 pairs of smaller ventral bristles; 5 pairs of lateral plantar bristles on each terminal tarsal segment but occasional specimens have 6 pairs.

Female: Length 3.6 mm, frontal tubercle less distinct than in the male, row of bristles on second segment of antennae exceeding the club; apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-2 respectively with some variation in different speci-

¹ There is a great variation in size of fleas of the same species. This and other measurements are from representative specimens and are not averages or means. They may disagree with published descriptions.

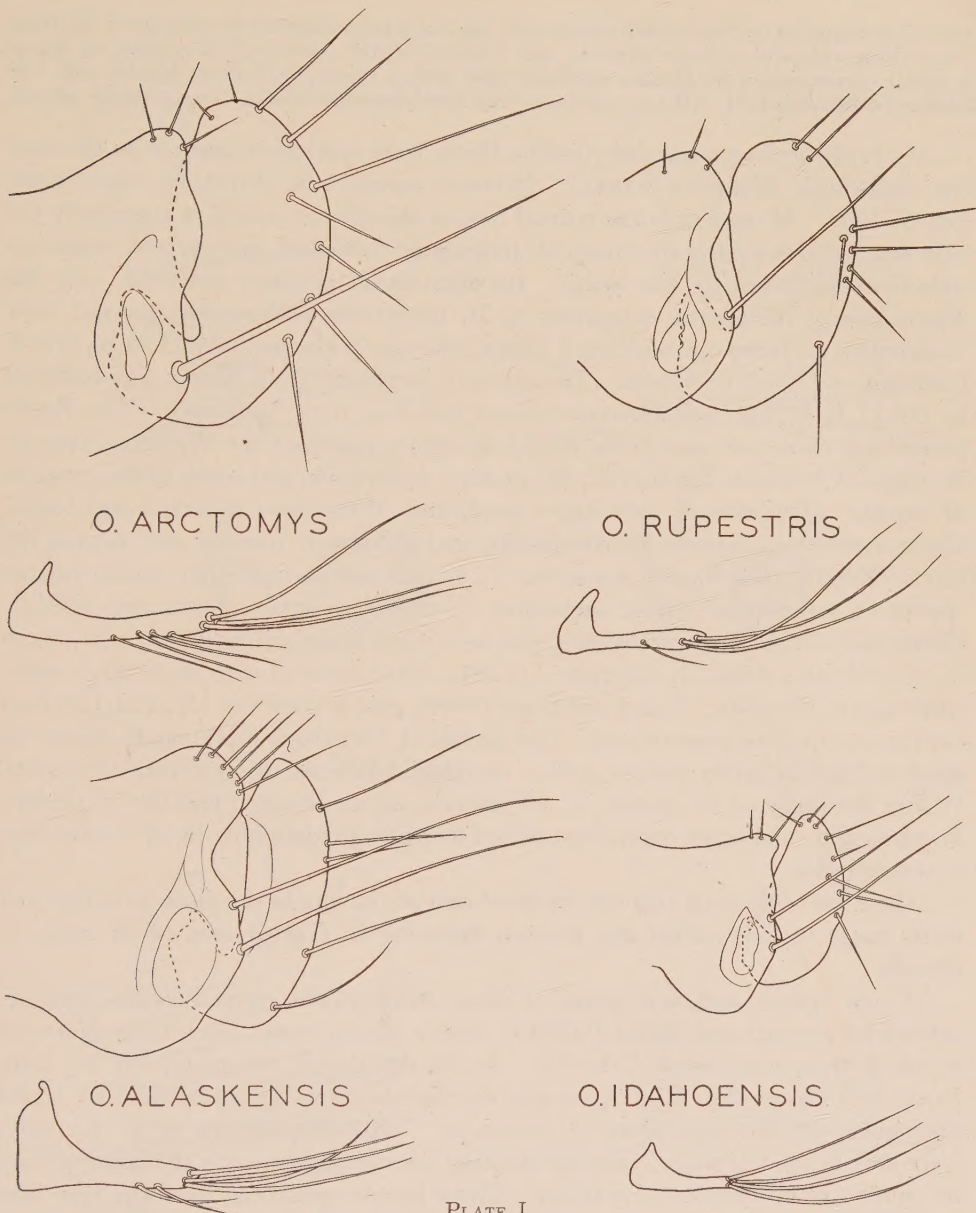


PLATE I

Claspers and Sternites VIII of Males.

SPECIMENS FIGURED IN PLATES I AND II.

Oropsylla arctomys (Baker):♂ from *Marmota monax*, Horseshoe Island, New York, June 4, 1932.♀ from *Marmota monax*, Gogebic Co., Michigan, June 30, 1920.*Oropsylla rupestris* (Jordan):♂ and ♀ from *Citellus richardsoni*, Calgary, Alberta, June 28, 1940. (Topotypes)*Oropsylla alaskensis* (Baker):♂ and ♀ from *Citellus parryi*, Thelon Game Preserve, Northwest Territories, Canada, June 24, 1937.*Oropsylla idahoensis* (Baker):♂ and ♀ from *Citellus columbianus*, Moscow, Idaho, June 1936. (Topotypes)

(The figures indicate a close relationship rather than striking differences in the four American species.)

mens, 3 antepygidial bristles on each side in most specimens but some with 4, stylet about $1\frac{1}{2}$ times longer than wide with a large terminal, and 3 lateral bristles, sternite VII rounded on dorsal posterior corner except in Alaskan specimens and with a row of 8-10 large bristles and 5-10 smaller bristles anteriorly, Alaskan specimens with very numerous bristles; receptaculum figured.

Oropsylla arctomys was described by Baker from specimens taken from the eastern woodchuck, *Marmota monax*, "*Arctomys monax*," at Peterboro, New York, July 9, 1900. *M. monax* is the normal host of this species and it is apparently the only flea found regularly on this host, judging by published records and numerous collections examined by the writer. Its distribution probably coincides with the distribution of the several subspecies of *M. monax* from Massachusetts and New York across northern central United States, through Michigan, Minnesota to British Columbia and north to Alaska. The series of specimens from Alaska was collected by Dr. C. B. Philip from *Marmota monax ochracea*, near Fairbanks. This flea is present but extremely rare in the Rocky Mountain region of the Western States in the range of *Marmota flaviventris*, the western woodchuck, and south of the range of *M. monax* which extends only into a small area of northern Montana and Idaho. These western woodchucks are consistently and abundantly infested with fleas of the genus *Thrassis*, including *T. acamantis*, *T. howelli* and several other closely related species or subspecies. These outnumber *O. arctomys* on *M. flaviventris* 1000-1. Collections of fleas from *M. monax* that have been examined usually contain from 1-10 specimens, while *M. flaviventris* is often found infested with 25 to 50 or more specimens of *Thrassis*. Eskey and Haas (1940) give averages of 13, 22 and 56 fleas per marmot in three western areas. No species of *Thrassis* is found on *M. monax* in eastern North America, nor on Asiatic marmots which are infested with *Oropsylla*. Within the range of *M. monax*, *O. arctomys* is also a common parasite of skunks, *Mephitis* spp., and other carnivores, which indicates predation or living in the dens of woodchucks.

There are no records available of fleas from *M. monax* in the most southern part of its range in the Central and Eastern States or in a great area of its range in Canada.

A new species and new genus of fleas, *Aetheopsylla septentrionalis*, was described by Stewart and Holland (1940) from a single female taken from *Marmota monax petrensis* in British Columbia. In the description the genus was not compared with *Oropsylla*. The figure and description show no characters that would distinguish the specimen from *O. arctomys*. Typical specimens of *O. arctomys* were sent to the Canadian National Museum for comparison with the holotype and no significant differences were noted. Three female specimens from the type host and type locality have been examined and are *O. arctomys*. It is the writer's opinion that *Aetheopsylla septentrionalis* is a synonym of *Oropsylla arctomys*. Mr. Holland concurs in this decision.

M. monax, parasitized by *O. arctomys*, is the only fossorial rodent of the family Sciuridae, a group so important in the epidemiology of sylvatic plague, that extends from the western plague infected area east to the Atlantic Coast. Plague has already been found in marmots, *M. flaviventris*, and in their parasites in western United States (Eskey and Haas, 1940). *O. silantiewi* (Wagner) and its several subspecies are the commonest flea parasites on *Marmota* in Asia (Ioff, 1925) where this mammal is an important plague reservoir. The pneumonic plague epidemics in

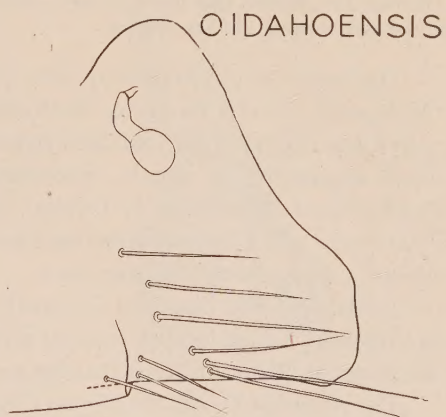
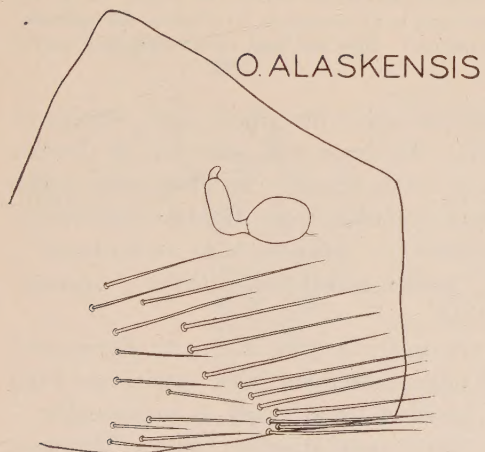
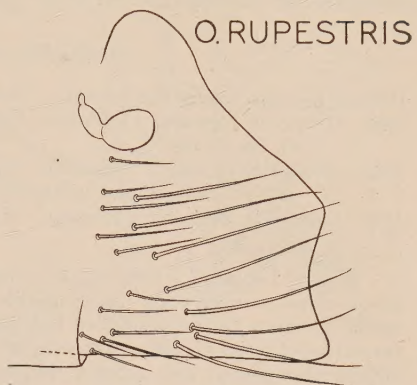
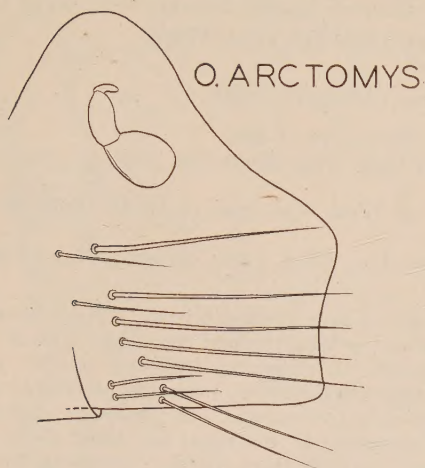


PLATE II
Receptaculi and Sternites VII of Females

Manchuria of 1910-1911 and 1920-1921 initiated among hunters who were taking marmots or "tarbagans" for their pelts (Wu Lien-Teh et al, 1936).

Oropsylla rupestris (Jordan) 1929

1936. *Oropsylla rupestris* (Jordan). Ioff, Zeit. Parasit. 9: 73-124.
 1940. *Oropsylla rupestris* (Jordan). Eskey and Haas, Pub. Health Ser. Bull. No. 254: 30, 40, 41, 58, 59, 64, 73.
 1942. *Oropsylla rupestris* (Jordan). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 120. (Bibliography up to 1939.)
 1943. *Oropsylla rupestris* (Jordan). Ewing and Fox, U. S. Dept. of Agric. Misc. Pub. No. 500: 50.

Male: Length 2.1 mm, frontal tubercle small, 2 pairs preoral bristles, 1 large bristle and row of small bristles along dorsal margin of antennal groove, pronotal ctenidium of 18-20 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-2 respectively, basal abdominal sternite with 1 pair ventral bristles, moveable process of clasper broadly club-shaped with anterior margin convex as in *O. arctomys*. In a topotype ♂ there are two unequal acetabular bristles well separated on each side, the upper one being about $\frac{1}{2}$ the length of the lower bristle. In all other specimens available there is only 1 acetabular bristle as figured by Jordan (1929), and as found in *O. arctomys*. Sternite VIII with 4 or 5 large sub-terminal bristles and 1 to 3 pairs of smaller ventral bristles.

Female: Length 3.1 mm, frontal tubercle small, pronotal ctenidium of 24 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 2-2-2-2 respectively, basal abdominal sternites with 1 lateral and a ventral pair of bristles; style $1\frac{1}{2}$ times longer than broad, with 1 terminal and 4 lateral bristles, sternite VII with a row of 8 large and 10 or 11 scattering smaller bristles, receptaculum figured. The most reliable character for the separation of *O. rupestris* from *O. arctomys* appears to be that mentioned in the original description by Jordan, namely the relative position of the spiracle and lower bristle on tergite II, in *O. rupestris* this bristle is definitely posterior to spiracle and in *O. arctomys* it is below the spiracle. (It must be admitted that this is a very minute character.) Size and host relationship are useful in the determination of the species.

The female of *Oropsylla rupestris* (Jordan) was first described as the allotype of *Opisocrostis labis* (Jordan & Rothschild) but the error was corrected by Jordan with a description of the male and redescription of the female. Its close relationship to *O. arctomys* was noted. Specimens were recorded from *Mustela longicauda*, (= "*Putorius longicauda*"), *Citellus richardsoni* (= "*Spermophilus richardsoni*"), *Thomomys* and *Canis* in Alberta, Canada. Jordan stated that *Citellus* ("*Spermophilus*"), is probably the true host.

Jordan (1937) recorded 2 females of *O. rupestris* from *Marmota flaviventris* in Colorado. This locality is so far from all other collections of the species and from the range of the normal host that the record, based on females only, is questionable.

The record of *O. rupestris* from *Citellus columbianus*, Missoula County, Montana, by Jellison, Kohls and Mills (1943) based on a single ♂ specimen must also be questioned as the location is distinctly west of the range of *C. richardsoni*.

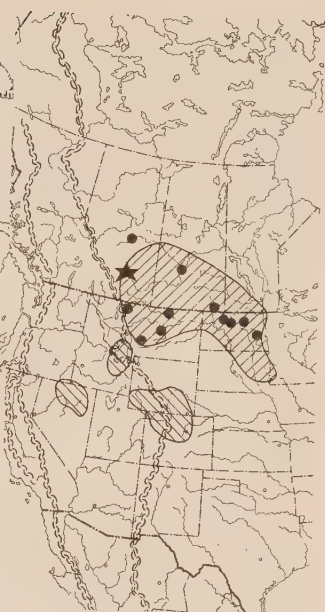
Further collections confirm Jordan's statement that this is a ground squirrel flea and furthermore its geographical distribution seems to be limited to one species of ground squirrel, i.e., *C. richardsoni*. All specimens examined by the writer from *Citellus*, where the host had been specifically determined, came from *C. richardsoni*, except the one ♂ specimen from *C. columbianus* noted above. It may also be found on *C. franklini* and *C. tridecemlineatus* where their ranges overlap the range of *C. richardsoni*, but present records indicate it has very strict host specificity.

The host relationships of this species involves a critical problem in mammal taxonomy. Howell (1938) regards the "Wyoming ground squirrel" as a subspecies of *C. richardsoni* and states that *C. r. elegans* and *C. r. richardsoni* intergrade in

O. ARCTOMYS



O. RUPESTRIS



O. ALASKENSIS



O. IDAHOENSIS

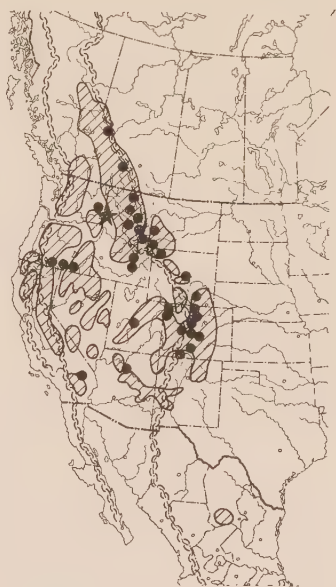


PLATE III

¹ Collection records of *O. arctomys* superimposed on distribution map of *Marmota monax*, after Anthony, 1928.

Collection records of *O. rupestris* superimposed on distribution map of *Citellus richardsoni*, after Howell, 1938. Large area = *C. r. richardsoni*. Small areas = *C. r. elegans* and *C. r. nevadensis*.

Collection records of *O. alaskensis* superimposed on distribution map of *Citellus parryii*, after Howell, 1938.

Collection records of *O. idahoensis* superimposed on distribution map of *Citellus (Callospermophilus) lateralis*, after Howell, 1938.

(A star indicates type locality of the species.)

southwestern Montana, whereas Davis (1939) and Anthony (1928) consider them as distinct species. The writer has experienced much difficulty in the specific identification in the field of ground squirrels in Beaverhead, Madison, and Gallatin Counties, Montana, where these two squirrels meet and possibly intergrade. All flea specimens from typical *C. r. richardsoni* in Gallatin County have been *O. rupestris*, although the series from this county includes a few atypical specimens. A large series of specimens from what is probably *C. r. elegans* in Madison and Beaverhead counties are *O. idahoensis* as are all collections from *C. r. elegans* in Wyoming.

It is very unusual for a species of flea to be restricted to one of two subspecies of hosts which intergrade in their overlapping territory. A more exact study of this parasite-host problem would be of interest.

O. rupestris was found to be an efficient vector of plague under laboratory conditions and plague was reported in its natural host *C. richardsoni* by Eskey and Haas (1940).

Plague infection has been reported in ground squirrel fleas from Alberta in 1942 (Public Health Reports) within the range of *C. richardsoni* but the squirrel host was not specifically given. An endemic area of sylvatic plague covering over 2000 square miles in southern Alberta is reported by Brown and Roy (1943). This area is within the range of *C. richardsoni* and it is assumed that this is the ground squirrel reservoir concerned.

Oropsylla alaskensis (Baker) 1904

1942. *Oropsylla alaskensis* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 118. (Bibliography up to 1939.)

1943. *Oropsylla alaskensis* (Baker). Ewing and Fox, U. S. Dept. of Agric. Misc. Pub. No. 500: 49.

Male: Length 3.7 mm, frontal tubercle very small, preoral bristles 2 pairs, labial palpi exceeding trochanters and about $\frac{1}{2}$ of femur, dorsal border of antennal groove with one large and many fine bristles; pronotal ctenidium of 22 to 24 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV with 1-2-2-1 respectively, 5 lateral plantar bristles on each terminal tarsal segment; basal abdominal sternite with 1 pair of ventral bristles only, antepygial bristles 1 large and 2 minute in each group. Two acetabular bristles on each side, well separated, moveable process of clasper long, anterior margin straight; sternite VIII with 4 large terminal and 2 pairs of medium ventral bristles.

Female: Length 4.6 mm, pronotal ctenidium of 24 to 26 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 2-2-2-0 in 1 specimen and 1-2-2-1 in another (this character is variable in many species); stylet two times longer than wide with one or two large terminal bristles and 4 lateral bristles, sternite VII not rounded at posterior dorsal corner, and with a row of 9 large and 10 small scattering bristles, receptaculum figured.

This species has been known only by the type collection which included both sexes from "*Citellus barrowensis*" = *Citellus parryi barrowensis* from Point Barrow, Alaska. This is the most northerly record of a ground squirrel flea in North America and the species is one of the largest of the ground squirrel fleas. Through the courtesy of Mr. Holland, I have seen the following specimens from the Northwest Territories, Canada:

From *Citellus*, Thelon Game Sanctuary, Northeast of Great Slave Lake, July 18, 1937, 1 ♂, 1 ♀, collected by C. H. D. Clarke.

From *Citellus*, Baker Lake, Aug. 29, 1937, 1 ♀, collected by C. H. D. Clarke.

From Arctic weasel, *Mustela arctica*, Baker Lake, Aug. 27, 1937, 1 ♀.

From Arctic fox, *Alopex lagopus inuitus*, Craig Harbour, Ellesmere Island, Nov. 17, 1934, 4 ♀ ♀.

The only species of ground squirrel in this general region, and the probable host of the flea, is *Citellus parryii parryii* according to a distribution map given by Howell (1938).

Besides the type specimens, there is also a single female of *O. alaskensis* without host data in the United States National Museum from Longitude 141°, Latitude 69°, collected by J. M. Jessup and J. M. Aldrich, August 14, 1912. This locality, near the Alaskan-Yukon border north of the Arctic Circle, is also within the range of *C. p. parryii*.

Oropsylla idahoensis (Baker) 1904 = *Ceratophyllus poeantis* Rothschild = *Ceratophyllus bertholfi* Fox

- 1936. *Oropsylla idahoensis* (Baker). Ioff, Zeit. Parasit. 9: 73-124.
- 1939. *Oropsylla idahoensis* (Baker). Jellison and Kohls, Pub. Health Rept. 54: 2022-2023.
- 1940. *Oropsylla idahoensis* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 118-120. (Bibliography and synonymy up to 1939.)
- 1940. *Oropsylla idahoensis* (Baker). Eskey and Haas, Pub. Health Bull. No. 254: 30, 32, 33, 39, 40, 41, 45, 57, 60, 64, 73, figs. 54, 55, 58, 61.
- 1940. *Oropsylla idahoensis* (Baker). Hubbard, Pac. Univ. Bull. No. 37(4).
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- 1942. *Oropsylla idahoensis* (Baker). Augustson, Bull. South. Cal. Acad. Sci. 40: 153-154.
- 1943. *Oropsylla idahoensis* (Baker). Augustson, Bull. South. Cal. Acad. Sci. 42: 82.

Male: Length 2 mm, frontal tubercle small but distinct, contrary to original description; 2 pairs preoral bristles, labial palpi exceeding trochanter and about equal $\frac{1}{2}$ of forefemur; pronotal ctenidium of 16-18 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-1 respectively, basal abdominal sternite with a pair of ventral bristles, sternite VIII small with 4 terminal bristles closely grouped and with 0, 1 or 2 pairs of smaller ventral bristles; moveable process of clasper with an obtuse angle on anterior margin not rounded as *O. arctomys* or *O. rupestris*; 2 acetabular bristles, well separated; 1 large and 1 very small antepygidial bristle, in each group.

Female: Length 2.7 mm, frontal tubercle small, sternite VII with a row of 6 large bristles and 3 or 4 smaller scattering bristles on each side, stylet tapering to a point $1\frac{1}{2}$ times longer than wide, and with 1 terminal and 4 lateral bristles.

Baker described both sexes of *Oropsylla idahoensis* from specimens collected from *Citellus columbianus* at Moscow, Idaho, and it has since been recorded from many species of *Citellus* (*sensu lato*) in the western part of North America. On *Citellus columbianus* it is found in association with and usually outnumbered by *Thrassis petiolatus* (Baker), *Opisocrostitis tuberculatus* (Baker), and *Neopsylla inopina* Rothschild. On most other species of ground squirrels it is likewise found in association with some species of *Thrassis*, *Opisocrostitis* and *Neopsylla*. However, as suggested by Hubbard (1940), it is the only consistent parasite on the golden-mantled ground squirrel, *Citellus (Callospermophilus) lateralis*. *C. lateralis* is host to stray fleas from *Eutamias*, *Tamiasciurus* and *Citellus*, and other rodents which share the same habitat but not with the same regularity that it is host to *O. idahoensis*. The range of this flea corresponds roughly with the range of *C. lateralis* except in the extreme north where *O. idahoensis* extends into Alaska on other species of *Citellus*, while *C. lateralis* extends only into British Columbia and Alberta. It exists consistently and certainly breeds in ground squirrel populations many miles from the habitat of *Callospermophilus* yet the general distribution of the parasite and this host are certainly related. This parasite is conspicuously absent from ground squirrels in the coastal region of California where *Diamanus montanus* is so abundant, and from ground squirrels in the prairie states east of the Rocky Mountains.

The collection of specimens available contains two series of special interest, one from *Citellus* in Alaska, and the other from *Cynomys* in Colorado. The Alaskan specimens are larger, darker and have more bristles and ctenidial spines than specimens from the type locality. Sternite VIII of the male in these specimens is also wider and has some additional bristles. Those from *Cynomys*, near the southern extremity of the range of the species are also large and robust specimens. As *Cynomys* is a distinct genus, related to both *Citellus* and *Marmota* and of similar habits a distinct species of *Oropsylla* might be expected on it. The series of fleas available does not warrant a separation of another species or subspecies but emphasizes the rather wide variation and wide geographical as well as host range of *O. idahoensis*.

O. idahoensis is very similar to *O. iloviaskii*, a parasite of *Citellus fulvus* of the Russian Steppes, and to *O. elana* from *Citellus* in Manchuria. There is little in the published figures or descriptions to separate the three species. A series of all three forms is necessary to determine their synonymy or identity.

SUMMARY

The American species of *Oropsylla*, namely *O. alaskensis*, *O. arctomys*, *O. idahoensis*, and *O. rupestris*, have been reviewed. Present records indicate the following host relationships and geographical distribution:

O. alaskensis is restricted to the ground squirrels of the *Citellus parryi* group of the Far North, even entering the Arctic Circle.

O. arctomys is a characteristic parasite of the woodchuck *Marmota monax*, but only very rarely occurs on other species of *Marmota* beyond the range of *M. monax*.

O. rupestris appears to be limited to *C. richardsoni richardsoni*.

O. idahoensis shows less host specificity and is found on a great many species of *Citellus* and also on *Cynomys* in the Western States, Canada and Alaska. It is the most characteristic flea parasite of the squirrel subgenus *Callospermophilus* and its range roughly coincides with this host except in the Far North.

All species are found occasionally on rodents other than their normal hosts and frequently on predators within their range.

ACKNOWLEDGMENTS

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Drawings were made by Gretchen Gayhart Jellison.

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EFFECTS OF VARIOUS SULFA COMPOUNDS ON THE PROTOZOAN PARASITE, *EIMERIA TENELLA**

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INTRODUCTION

Following the work of Horton-Smith and Taylor (1942) on the use of sulfamethazine and sulfadiazine in the control of caecal coccidiosis in chickens, the present authors endeavored to confirm and extend the information gained from their work. To do this a series of twenty-five experiments involving the use of several hundred birds was devised.

Since sulfadiazine was more readily available than was sulfamethazine at the time these experiments were conducted, emphasis was placed on the former compound.

MATERIALS AND METHODS

The laboratory animals used were Single Comb White Leghorn chickens which were furnished by the Department of Poultry Husbandry at the University of Wisconsin. Their ages ranged from 2 days to 2½ months. The sexes of these chickens were approximately equal in number and the data were recorded separately as to sex but as no significant difference was seen, the data from both sexes in each experiment were combined in the final consideration.

The birds used were all kept in a separate house (Edgar and Herrick, 1944) and fed with sterilized feed (Fish, 1931) until needed. At this time they were removed to another building and immediately infected. Following infection they were kept under conditions as described by Herrick, Ott and Holmes (1936), i.e., sterile cages supplied with sterile feed and running water. The method of infection and the oöcysts used were as described by Edgar and Herrick (1944).

The chickens were distributed to their respective experimental cages as they were selected at random, thus making the weight and physical condition of the birds in the various cages practically identical.

The severity of the infection and the therapeutic value of the treatment of the birds was evaluated in four ways: (1) daily observations of clinical symptoms, (2) weight changes from the beginning to the end of the experiments, (3) feed consumption, and (4) the number and size of the caecal lesions at autopsy (Herrick, Holmes and DeGiusti, 1942).

Administration of sulfadiazine was by: (1) 0.5-gram pills, (2) aqueous suspension of the powdered form, (3) powder in appropriate sized gelatin capsules, and (4) mixed in the feed. The sulfadiazine suspension was prepared by mixing 100 grams of the powder with 250 cc. of distilled water. It was then administered into the crop by means of a calibrated syringe, care being taken that each bird received its proper dose. The amount of the compound given was dependent upon the weight of the bird since they were treated at the rate of 0.0128 grams of sulfadiazine per gram weight of bird.

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To determine the number of oöcysts which were produced and sporulated after treatment of the chicken with sulfadiazine, the oöcysts were concentrated by means of the modified Lane technique (Lane, C., 1923–1925). Fifty high-power (4-mm) objective fields of the supernatant in the tubes used in the concentration process were examined by placing a drop of the material on a regular glass microscope slide. Counts were then made of the number of sporulated and unsporulated oöcysts, gametocytes and imperfect forms; the results were recorded separately. From these figures the percentage of sporulation of the oöcysts was found. In those cultures showing no oöcysts on first examination, two more slides of the same supernatant fluid were examined to insure that no forms had been missed.

PRESENTATION OF DATA

The data on administration of sulfadiazine will be presented under two main headings: (1) single dosage and (2) in the feed. Prevention, cure and sporulation effect will be considered in that order. Observations on other data and on the use of other compounds will be noted as addenda.

In preliminary work to determine the minimum lethal dose of sulfadiazine for chickens, it was found that they had considerable tolerance for the compound. As much as 12 grams were given to birds weighing from 400 to 600 grams without causing death. The salient feature of this test was in finding that sulfadiazine, when given in large doses, resulted in a decided and rather rapid weight loss. The crop contents became more solidified than in normal birds, and the animal apparently starved due to an inability to carry through the normal digestive processes.

In subsequent experiments it was found, concomitantly with the aforementioned starvation, that the caecal pouch contents were quite abnormal both in consistency and in color. Normally the caecal pouch content is a finely granular substance containing many fat droplets when seen under the microscope and teems with bacteria of a wide variety. After treatment with sulfadiazine the pouch contents were moderately coarse and flaky and of a darker brown color than normal. The bacterial flora was greatly reduced in total number and the most conspicuous organism was a short, thick, motile bacillus. No attempt was made in these experiments to identify the bacteria found.

To determine the optimum dose to be used in subsequent trials, the sulfadiazine was administered in varying amounts to seven groups of birds, the lowest level being 0.0004 gm of sulfadiazine per gram weight of bird and this dosage was increased in multiples of two to each succeeding group. The maximum level used was 0.0256 gm; the optimum was found to be 0.0128 gm.

PREVENTION BY SINGLE DOSE

Having found a wide margin of safety in treatment, an experiment was devised to determine how long a single dose of 0.0128 grams of sulfadiazine per gram weight of bird would be maintained at a protective level in birds of from 400 to 600 grams weight and what effect such treatment might have on their weight changes.

The birds used in this experiment were divided into nine groups, two of which were used as (1) infected, untreated controls and (2) uninfected, untreated (fully normal) controls. The other seven groups were given the sulfadiazine for varying lengths of time before infection. One group was treated 6 days prior to infection, one 5 days before infection and so on until that group was reached which was given

the sulfadiazine and the infection at the same time, the "zero-day" group. At the time the zero-day group was infected, all other groups were also infected except the fully normal controls. Each bird was given 400,000 oöcysts. Seven days later the birds were all killed to determine those which had resisted infection.

As seen in Table 1, none of those birds treated on zero-day took the infection. Of those treated 1 full day before infection, one-half were negative and one-half were severely infected. In the group treated 2 full days prior to infection, 1 bird was negative, 1 had a 2-plus infection and 2 had a 4-plus infection. All the chickens treated 3 or more days before being given oöcysts developed severe caecal lesions as did the infected, untreated controls. The fully normal birds were all negative. It seems, therefore, that the sulfadiazine in large doses remained at a fully protective

TABLE 1.—*Illustrating the length of time a single dose of 0.0128 grams of sulfadiazine per gram weight of bird remains effective as a preventive against 400,000 oöcysts of E. tenella and how it affects the weight gains of the treated chickens*

Use of bird	Group No.	Severity of infection	No. of birds used	Average initial weight	Average terminal weight	Average daily gain	No. of days treated with sulfadiazine before autopsy*
				gm	gm	gm	
Uninfected untreated (fully normal) control	1	—, —, —	3	596.6	833.6	17.9	0
Infected untreated control	2	4+, 4+, 4+	3	586.0	629.6	3.3	0
"0" days	3	—, —, —, —	4	553.5	653.0	14.6	7
1 day	4	—, —, 4+, 4+	4	567.0	655.0	10.9	8
2 days	5	—, 2+, 4+, 4+	4	565.7	661.7	10.6	9
3 days	6	+, 3+, 4+, 4+	4	455.7	502.7	4.7	10
4 days	7	2+, 4+, 4+, 4+	4	596.0	643.0	7.0	11
5 days	8	4+, 4+, 4+	3	545.0	594.0	4.1	12
6 days	9	3+, 4+, 4+, *	3	508.3	522.6	1.1	13

* Autopsied 7 days following infection.

level for a comparatively short period of time. Treatment and infection at the same time, zero-day, was the only time of treatment which could be depended upon to prevent the infection. In the birds treated 1 day before infection the protection was not constant. In those treated 2 days before infection the protection was doubtful, and in those treated 3 or more days before infection, no protection could be demonstrated. Protection by sulfadiazine is probably effected by an attack on the sporozoites of *E. tenella* when they are released from the oöcysts.

Comparing the weight changes of the different groups (Table 1), one can see a general decline in the average daily gains from those birds having had sulfadiazine for only 7 days, the zero-day group, to those having had it for 13 days, the 6-day group. It seems that the difference in weight gains can be attributed to the effect of the sulfadiazine. It was most noticeably seen between the zero-day group, the infected controls, and the 6-day group. The weight gain difference between the infected and the uninfected controls is obviously due to the infection. The protocol of this particular experiment precluded the possibility of following the weight changes for a longer time period. It was believed, however, that the failure to gain weight continued with extended time until the weight changes were in the negative values. Rough experiments which followed substantiated this view. Birds tested on this

point did lose weight for a variable length of time but given sufficient time (several weeks) were able to overcome entirely the effect on their weight and rival the weight of the fully normal control birds.

THERAPEUSIS BY SINGLE ADMINISTRATION

Having determined the protective capacity of sulfadiazine against infection with *E. tenella*, an attempt was next made to establish its therapeutic value. To accomplish this end an experiment was set up on much the same plan as that used to demonstrate protection. A number of 2-day-old birds were divided into nine groups one of which acted as the fully normal controls. The remaining eight groups were infected at zero days with 50,000 sporulated oöcysts. Treatment was with a single 0.5-gram tablet of sulfadiazine and was instituted at the time of infection, zero-days, for group 1, 9 hours following for the second group and so on until the number 5 group was treated with the sulfadiazine 3 days after the oöcysts had been adminis-

TABLE 2.—Illustration of the comparative efficacy of sulfadiazine in single doses when given at various times following infection in curing coccidiosis in chickens 7 days old

Group number	Time of treatment in relation to time of infection ^{tt}	Number of birds used	Severity of lesions					
			—	1+	2+	3+	4+	Others
1	At the same time ^t	10		2	4	3	0	1*
2	9 hrs. post-infection ^t	10	2	1	1	0	6	0
3	1 day following ^t	10	1	1	2	4	0	2†
4	2 days following ^t	10	1	3	4	1	0	1*
5	3 days following ^t	10	8	1	0	1	0	0
6	4 days following ^t	10	3	4	3	0	0	0
7	5 days following ^t	8	1	1	2	4	0	0
8	0.5 gm pill daily for 5 days	10	6	4	0	0	0	0
9	Infected untreated controls	10	0	5	2	3	0	0
10	Fully normal controls (uninfected untreated)	8	8	0	0	0	0	0

† Dead from causes other than coccidiosis.

* Lost wing bands.

^t Given a single dose of 0.5 gm of sulfadiazine.

^{tt} Given 50,000 oöcysts.

tered. Group 8 was treated with 0.5 gram of sulfadiazine at the time of infection and each day thereafter for 5 days. Two days following the last treatment with the compound (7 days following infection), all the birds were autopsied in order to learn the therapeutic effect of the sulfadiazine as indicated by the severity of the caecal lesions.

By consulting Table 2, it can readily be seen that those birds treated on the third day following infection were the ones afforded the greatest protection by a single dose. Treated statistically, these data indicate that a cure of over 80% was effected by treatment on this day. It is questionable whether those birds treated at any later time were benefited by the treatment. In later experiments in which 1% sulfadiazine was mixed in the feed, each bird actually got about twice as much sulfadiazine per day as those getting a single 0.5-gram tablet per day. The failure in the zero-day group (group 1, Table 2) to resist infection must be ascribed therefore to the low level at which the sulfadiazine was given. As shown in Table 2, group 5 received 0.5 gram of sulfadiazine on the third day following infection and the life cycle of the parasite was disrupted in the first generation merozoite stage. Likewise group 8, which received 0.5 gram daily for 5 days, were benefited by inhibiting the same stage.

However, when this amount of sulfadiazine was given either at the time of infection or 9 hours following infection, when the sporozoite stage was present, little benefit was afforded. Since the sporozoites and the merozoites were both subjected to the same level of the drug and the sporozoites persisted while most of the merozoites were killed, it indicates that the sporozoites have a higher threshold of tolerance than do the merozoites. This is, however, not so surprising since in other sporozoan infections (malaria), particular stages of the parasite are more readily attacked by certain chemicals than are other stages.

SPORULATION

During routine autopsy, the caecal pouch tissues of several birds that had been treated with sulfadiazine during the sixth day of infection were examined and the caecal pouch contents were set away to sporulate (see Materials and Methods) for 24 hours. Contrary to expectation, no gametocytes or unsporulated oöcysts could

TABLE 3.—*Demonstrating the effects of a single large dose of sulfadiazine on production of and sporulation of oöcysts when given after the symptoms of coccidiosis appeared*

No. of birds used	Use of birds	No. of oöcysts counted	No. of oöcysts sporulated	No. of gametocytes or other forms counted	Percentage of sporulation	No. of high-power (4-mm obj.) fields counted	Average dose of sulfadiazine given
9 a*	Test	0	0	29	0.0	25	gm 9.9
1 a	Infected Control	360	268	92	80.0	14	0.0
8 b†	Test	0	0	10	0.0	50+	10.4
2 b	Infected Control	369	286	83	77.5	25	0.0
8 c††	Test	97	52	45	53.0	25	8.8
2 c	Infected Control	154	125	29	80.0	25	0.0
3	Fully normal Control	0	0	0	0.0	25	0.0

* Treated with sulfadiazine 6 days following infection.

† Treated with sulfadiazine 6 & 7 days following infection.

†† Treated with sulfadiazine 7 days following infection.

be found either in the scrapings of caecal epithelium or in the caecal contents. After allowing the caecal pouch contents to sporulate for 24 hours, a rigid examination was made of the culture after concentrating any oöcysts present by the modified Lane technique (l.c.). No form of the parasite could be found in the test birds, whereas in the normally infected control group not having had treatment, there were between 10 million and 15 million oöcysts per cc of the material brought to the surface. The counts were made by dilution using the Spencer Bright-Line Haemocytometer. Examination of the birds treated with sulfadiazine at the end of the sixth day showed normal numbers of oöcysts with a fairly high percentage of sporulation. The same was true of birds treated with sulfadiazine during the fifth day of infection. These data, considered in the light of work done by Edgar, Herrick and Fraser, 1944, on the development of *E. tenella*, pointed to the possibility of the sulfadiazine attacking the parasite when it is free as the second generation merozoite in the lumen of the caecal pouch. With this lead, more trials were conducted to test the efficacy of sulfadiazine in preventing the formation of oöcysts after the infection had become firmly established.

Uninfected birds 88 days old were infected with 50,000 sporulated oöcysts and then divided into three groups. Controlling these groups were an infected untreated

control group and a group of fully normal birds, i.e., untreated and uninfected. All groups to be given treatment were dosed with 0.0128 gram of sulfadiazine per gram weight of bird. Group 1 was treated on the sixth day following infection, the second group was given its treatment on the sixth and seventh days, and the third group was treated on the seventh day of infection only. The compound was given as a single dose except in group 2 where one-half of the full treatment was given each of 2 successive days.

The infection was established and the second generation merozoites escaped from the host cells as was evidenced by the copious hemorrhage on the fifth day. By the seventh day of infection these symptoms had disappeared and all of the birds recovered. Ten days from the time of infection all of the birds were killed for examination of caecal lesions and sporulation of any oöcysts that might be present in the caecal pouch contents.

As shown in Table 3, those birds given sulfadiazine at the beginning of the sixth day and also the group treated on the sixth and seventh days produced a small number of oöcysts and gametocytes, neither of which were capable of sporulation. Those birds treated on the seventh day only and the infected, untreated controls, produced a fair number of oöcysts which sporulated. The fully normal controls showed no forms whatsoever, of *E. tenella*.

From this it can be concluded that the sulfadiazine exerts its influence on the second generation merozoites, and possibly on the developing gametocytes as well. Cytological inquiry is in progress on this latter point. It can be said that treatment with sulfadiazine as late as 5 days and 22 hours following infection, when the bird was suffering severe caecal hemorrhages, resulted in failure of the parasite, *E. tenella*, to complete its life cycle. In the few cases where oöcysts were produced, they did not sporulate.

SULFADIAZINE IN THE FEED

Finding individual treatment with sulfadiazine too expensive of time, it was decided to try using various percentages of the compound in the feed and still accomplish the same ends as those produced by single doses. The purpose was to determine: (1) the level at which it could be given without causing excessive deleterious effects, (2) its protective capacity, (3) its therapeutic value, and (4) its effect on production and sporulation of oöcysts. To determine these points, several experiments were set up.

To learn the best protective level of sulfadiazine, 82 birds averaging 198 grams each were given various percentages of the compound in their feed. There were 7 groups in all, the infected untreated controls, the uninfected untreated (fully normal) controls, those having 1%, 0.5%, 0.25%, 0.125% and 0.062% of sulfadiazine in the feed in the respective groups. Twenty-four uninfected birds were distributed equally among the cages getting the respective levels of sulfadiazine so as to have uninfected treated controls for each level. All save the uninfected control birds were given 300,000 sporulated oöcysts immediately after starting them on the treatment. Sulfadiazine was continued in the feed for the duration of the experiments, i.e., 7 days following infection, at which time the birds were autopsied. The results of this experiment, as shown in Table 4, indicate that group 1 (1% sulfadiazine) was fully protected, group 2 (0.5% sulfadiazine) was one-half protected and one-half took a light infection. In group 3 (0.25% sulfadiazine) 6 had severe caecal lesions, 1 had moderate lesions and 1 was negative. Levels of the compound of 0.125% or below

allowed moderate to severe infections in all cases. None of the uninfected controls became accidentally infected. This demonstrated the unreliability of levels below 1% in ability to protect and that 1% afforded complete protection from infection with *E. tenella* when treatment began before or at the time of infection.

Next an experiment was set up to learn if levels of sulfadiazine higher than 1% would be injurious. In this test, birds 42 days old and averaging 206.3 grams each were divided at random into 8 groups (see Table 2). Infection was with 160,000 sporulated oöcysts, the infectivity of which had been previously tested.

TABLE 4.—*Illustrating the protection afforded chickens against infection by E. tenella with varying amounts of sulfadiazine*

Per cent of sulfadiazine used	No. of birds used	Birds used as	Time (in hours) when sulfadiazine was given			Pathology of caecal lesions
			Before infection	After infection	Total time (hr.) of being fed sulfadiazine	
1.0	10	Test	0	0	168	10 neg.
	8	Test				8 neg.
	4	Treated uninfected	24	168	192	4 neg.
0.5	7	Test				4 neg.
	4	Treated uninfected	24	168	192	4 neg.
0.25	8	Test				4-, 4-, 4-, 4-, 4-, 4-, 2-, -
	4	Treated uninfected	24	168	192	4 neg.
0.125	8	Test				2-, 2-, 4-, 4-, 4-, 4-, 4-, 4-
	4	Treated uninfected	24	168	192	4 neg.
0.062	8	Test				2+, 2+, 4+, 4+, 4+, 4+, 4+, 4+
	4	Treated uninfected	24	168	192	4 neg.
0.0	8	Infected but not treated				All (8) 4+
	4	Uninfected untreated (fully normal)	0	0	0	All negative

Treatment with sulfadiazine was started in the 3 test groups and their infected cage mate controls, at 1%, 3%, and 5% levels, respectively. This treatment began 5 days after giving the oöcysts and was continued for 5 days thereafter when the birds were autopsied. The severity of caecal infection was then noted and the caecal pouch contents were put aside for 24 hours to allow any oöcysts present to sporulate. Weights of each bird were taken 1 day before infection, at the time of treatment, and on the tenth day (at autopsy).

The data from this experiment are consolidated in Table 5 where the gross pathology, the weight changes and the effect on the production and sporulation of

TABLE 5.—*Illustrating the effect of sulfadiazine on E. tenella when incorporated in the feed during the fifth and sixth days following infection**

Use of bird	No. of birds	Group No.	Severity of infection	Daily av. wt. chg. dur. 1st 5 days	Daily av. wt. chg. 2nd 5 days	Daily av. wt. chg. 10 days	Percentage of sporulation of oocysts	No. of oocysts	Deaths between 5 & 10 day infect.
Fully normal control	8	1	All neg.	gm. 3.6	gm. 5.55	gm. 4.6	0	0	0
Infect. un-treat. controls	4	2	4+, 4+, 4+, 1+	5.8	-3.5	1.15	75%	Many	0
1% feed uninfected	3	3	All neg.	7.4	5.5	5.5	0	0	0
1% feed infected	7	4	4+, 3+, 3+, 3+, 4+, 4+, 4+	4.6	2.4	3.14	0	0	(28.5%) 2
3% feed uninfected	4	5	All neg.	8.3	-3.55	2.1	0	0	0
3% feed infected	6	6	4+, 3+, 4+, 4+, 4+, 4+	8.3	-7.0	1.3	0	0	(50%) 3
5% feed uninfected	3	7	All neg.	6.0	-5.7	+0.23	0	0	0
5% feed infected	7	8	4+, 4+, 4+, 4+, 4+, 4+	2.54	-4.4	-0.85	0	0	(71%) 5

oöcysts are noted. All birds given the infection showed typical symptoms whereas those not given oöcysts were negative. The sulfadiazine in none of the three percentages used can be said to have a measurable value in alleviating the symptoms of the developing disease but no oöcysts could be found in the caecal pouch contents of any of the treated birds. In the untreated birds, on the contrary, a large number of oöcysts were present in the caeca and these oöcysts sporulated normally. Although the sulfadiazine prevented the production of oöcysts, it can be seen (Table 5) that treatment with the higher percentages (3% and 5%) resulted in more severe weight losses than were caused by the infection alone. This is in accord with the work of Schmidt et al, 1944. It can be concluded, then, that the higher levels did adversely affect the weight gains of the birds and that none of the levels had any visible effect on the clinical symptoms of the disease but all concentrations did prevent the production of oöcysts. It is conceivable that if the sulfadiazine is given even after the appearance of blood in the faeces, it may be of considerable value in preventing an epidemic. This would be accomplished by preventing infection in those not infected and by preventing those already infected from becoming carriers.

After it was discovered that sulfadiazine prevented the development of oöcysts, the question arose as to the ability of the compound to prevent the sporulation of oöcysts after they had already become formed in the caecal epithelium. To answer this question, a group of birds was given a sub-lethal dose of oöcysts and the infection was allowed to proceed for 13 days. At this time all the birds were passing a considerable number of oöcysts. The group was then divided in half, one-half being given 1% sulfadiazine in its feed, and the other half continued on normal sterile feed. After feeding the one group sulfadiazine for 3 days, all the birds were killed and their caecal pouch contents sporulated. The ensuing oöcyst count revealed no significant difference between the test birds and their controls either in the total number of oöcysts produced or in the percentage of their sporulation. From this and other related tests it was assumed that once the oöcyst had formed, it was no longer subject to the action of sulfadiazine.

IMMUNITY

In order to determine the rôle of oöcyst formation in development of resistance to subsequent infection, the following experiment was set up:

The birds used were divided at random into 3 groups: (1) the fully normal controls, (2) the infected untreated controls, and (3) the infected test birds. The test birds and the infected controls were each given 50,000 oöcysts. Treatment with 1% sulfadiazine was initiated in the test birds at the beginning of the sixth day and was continued for 24 hours thereafter at which time the feed containing sulfadiazine was replaced by normal sterile feed. The infected untreated controls and the fully normal controls were kept together after the fifth day of infection.

Fifteen days following the first infection with 50,000 oöcysts, a second infection of 200,000 oöcysts was given to all the birds. Six days following the second infection, all the birds were killed in order to examine the caecal lesions which were used as an index of the presence or absence of an immunity to *E. tenella*.

The resulting data (Table 6) showed the severity of infection from the second dose of oöcysts to be almost the reverse in the 3 groups as that which was produced by the first infection. The former fully normal controls all had very severe infections, the former infected controls ranged from a heavy infection to negative and the

TABLE 6.—Illustrating that gametogeny was not an important factor in the development of resistance to subsequent infections with *E. tenella*

Group	No. of chicks	Treatment at time of 1st infection with 50,000 oöcysts	Symptoms or lesions from 1st infection	No. of oöcysts given at second infection	Caecal lesions from second infection	Average daily wt. gains in gm over 21 day period	Days from beginning of experiment to autopsy
1	5	Uninfected untreated	Symptoms	200,000	1-3+ 4-4+	6.7	21
			None				
1A	6*		Lesions				
			None				6
2	6	Infected untreated	Symptoms	200,000	1-neg. 4-1+ 1-3+	8.0	21
			Medium to severe				
			Lesions				
2A	6*		1-2+ 1-3+ 4-4+				6
3	6	Infected treated	Symptoms	200,000	2-neg. 1-1+ 3-2+	9.0	21
			Medium to severe				
			Lesions				
3A	6*		1-2+ 5-4+				6

* These birds were autopsied on the 6th day of infection to indicate the probable severity of the initial infection in those chickens given a second infection.

sulfadiazine test birds ranged from a moderate to a negative infection. From these data it was concluded that the development of resistance to infection with *E. tenella* was not a function of the gametogenous phase in the life cycle of *E. tenella*.

OTHER COMPOUNDS TESTED

Believing sulfadiazine to be peculiar in its ability to entirely protect a bird from infection, to arrest the development of the infection on the third day and again at the beginning of the sixth day, several other compounds were tested in a like manner for the same properties. These compounds were sulfathiazole, sulfaguanidine, sulfonilamide, succinyl-sulfonilamide, lime sulphur, tetraethylthiuram monosulphide and barium antimonyl tartrate. Of these, sulfathiazole showed the most promise of comparing favorably to sulfadiazine when treatment was started at the time of infection. Accordingly, it was given further tests at 1% and 2% levels in the feed. It proved to be able to prevent the infection at either level when given from the time of infection onward for 10 days, the duration of the experiment. When given after the infection had once started, however, it failed to stop the course of the disease.

The other sulfa compounds tested showed little promise of having any closely comparable properties to those found in sulfadiazine. Because of this, further experiments were not carried out with them.

The barium antimonyl tartrate was suggested by a worker in the field since some of the tartrates are used in other protozoan infections as well as in some helminth infections in fowls. It was given as single doses and at various percentages in the feed but did not prevent infection in either case. In all the tests it proved far too toxic for further consideration.

Herrick, Holmes and DeGiusti (1942) found tetraethylthiuram monosulphide to have protective properties against *E. tenella* infection in the chicken with a 20:1 margin of safety in dosage. The experiments in this series, however, showed the compound to have a much smaller margin of safety. It was difficult to give a dose small enough in young chicks to avoid killing them within a day or two. This can probably be explained on the basis of the increased age of the compound. Aging seems to decrease its protective power and increase its toxicity.

SUMMARY

1. Sulfadiazine when given at the time of infection was an effective preventive when given as 1% of the feed or in single large doses.
2. When given during the third day of infection it materially reduced the severity of infection.
3. Given during the fifth and six days of infection it prevented oöcyst production.
4. At 2% or 3% levels in the feed it had a marked deleterious effect on the experimental animals.
5. It remained at a protective level in the chicken for not more than 1 day when given as a single large dose.
6. It did not influence the viability of the oöcysts after they had once formed in the tissues of the host.
7. Preventing the development of oöcysts did not influence the development of immunity.
8. Sulfathiazole prevented infections when given before or at the time of infection but had no effect after the sporozoites had become established.

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THE LARVAE OF *EUSTRONGYLIDES IGNOTUS* IN *FUNDULUS HETEROCLITUS*

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Eustrongylides larvae occur in *Fundulus diaphanus* (Chapin, 1926; Mueller, 1934), in *Fundulus heteroclitus* (von Brand, 1938), as well as in a variety of other fish (Hunter, 1937, 1942; von Brand, 1944). It is not possible to decide at the present time whether they all belong to one species, since the larvae of the various species are extremely similar. Von Brand and Simpson (1944), however, succeeded in bringing one specimen isolated from *Fundulus heteroclitus* to molt *in vitro*, and this worm was definitely identified as *Eustrongylides ignotus*. *Fundulus* is heavily infected with this parasite in the vicinity of Baltimore and it appeared worthwhile to study the occurrence of the worm in this fish on a large amount of material.

MATERIAL AND METHODS

The fish were procured between September 1941 and March 1942 from two bait fishers who caught *Fundulus* in large numbers from Chesapeake Bay in the immediate vicinity of Baltimore, Md. These dealers had usually, with the exception of January and February, large stocks of freshly caught fish at hand from which representative samples were purchased and brought to the laboratory where they were kept in aquaria until dissected. Due to the fact that *Fundulus heteroclitus* was most easily obtained, the investigation was restricted to this species. It is admitted, however, that some *Fundulus macrolepidotus* may have slipped in. These two species are extremely difficult to distinguish, but it is not believed that serious errors resulted from this possibility, since *Fundulus macrolepidotus* is often not considered as a distinct species, but only a variety of *Fundulus heteroclitus*.

All the dissected fish were classified as to sex and size, the latter by measuring them from the tip of the snout to the end of the caudal fin. The sex was determined by inspection of the gonads; in very small fish the gonads look macroscopically very much alike, but it was always possible to come to a decision with the help of a microscope. In so far as the parasites were concerned, notation was taken from their number in infected fish, their location in various organs and of their length. Because of the elasticity of the worms these measurements were somewhat difficult. The centimeter rule was always kept moist in order to insure against the worm adhering to it when extended. After being stretched on the rule by means of two forceps, it was allowed to contract until it just began to deviate from a straight line. This was taken as its normal length. All measurements, both of the host and parasite, were made to the nearest tenth of a centimeter.

RESULTS

A total of 3,507 fish was examined. Of these 465 (13.3%) were infected. Out of the total, 1589 (45.3%) were males, 1918 (54.7%) females. Parasites were

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found in 208 males (13.1% of the total males, 44.7% of the parasitized fish) and in 257 females (13.4% of the total females, 55.3% of the parasitized fish). There exists, therefore, no sex difference in the degree of parasitization.

In order to study whether a relation exists between the size of the fish and the size of the parasites, the fish were divided into size classes with the difference of 1 cm between the classes. Table 1 shows the relationships between the average size of the parasites and the size of the fish on the one hand, and the percentage of infected fish in each class on the other. It is obvious that smaller fish have smaller parasites. The average size of the worms remained unchanged only when the fish reached a length of about 9 cm. The growth rate of *Fundulus heteroclitus* has not yet been studied; it is, therefore, not possible to correlate these findings with the age of the fish. Dr. S. Hildebrand (personal communication) considers a *Fundulus* of about 5 cm as about 1 year old, although considerable variations in growth due to environmental conditions seem to occur. But it appears on the whole obvious that younger fish have on the average smaller parasites. This seems to indicate rather definitely that the worms grow over a rather long period, perhaps a year or two.

TABLE 1.—Relation between size of *Fundulus*, degree of parasitization and size of worms

Length of fish, cm	Average length of worms, cm	No. of positive fish	No. of negative fish	Percentage of parasitism
3-4	4.5	11	247	4
4-5	6.9	72	730	9
5-6	7.0	98	815	11
6-7	8.1	74	617	12
7-8	8.5	105	524	17
8-9	9.4	70	142	33
9-10	10.5	18	14	56
10-11	9.2	3	3	50
11-12	10.6	2	0	100

But even if the age of the fish were exactly known, it would not be possible to draw very definite conclusions about the growth rate of the parasite from field observations alone. The last column of Table 1, showing the percentage of infected fish indicates that the larger the fish become, the greater is the degree of parasitization. This remains true, even if we disregard the two largest size classes for which only a negligible number of fish was available. The entire situation indicates that fish of various size may contract infections and that, apparently, no age immunity develops.

Another possible interpretation for the observation that smaller fish harbor on an average small parasites, could be found in the assumption that the former do not afford sufficient space or food to allow the same growth of the parasites as larger fish. This, however, seems not likely, because some small fish have very long parasites; for example a worm 10.5 cm long was found in a fish of 4.2 cm; in another instance a 14-cm worm occurred in a 5-cm fish. It seems most likely that these fish acquired their infection at an extremely young age.

The question whether the incidence or the size of the parasites varies at different seasons cannot be decided with the material available; a study extending over several years would be required.

Of some interest are the observations made on the occurrence of multiple infections (Table 2). By far the greatest majority of the infected fish harbored only one worm, but two or three parasites in one host were not too rare. The maximum

TABLE 2.—Number of single and multiple infections

No. of worms per fish	No. of fish	Percentage of total infected fish
1	352	75.7
2	60	12.9
3	30	6.5
4	11	2.4
5	4	0.9
6	1	0.2
7	2	0.4
10	3	0.6
13	1	0.2
25	1	0.2

was 25 worms in a single fish. Multiple infections were encountered in every size group of fish, but the available material is not large enough to decide whether their frequency varies with the size of the host. The length of the parasites, in cases with two or three worms in one fish, was usually fairly close. A few examples may illustrate this: two worms recovered from one fish measured 10.2 and 14.0 cm; or in another case 1.3 and 2.7 cm. Exceptions, however, occur. Thus, the length of three parasites found in one host varied in one case between 1.3 and 7.0 cm, or in another case between 2.0 and 9.2 cm. In the heavier multiple infections the size range of the worms was usually wide. In the fish having 25 parasites, the smallest one measured 2.9, the largest one 13.5 cm. The most probable explanation for these observations is that the infections were contracted at the same time in those cases where the parasites had about the same length, while superinfection must be assumed when large differences in size did occur. If this explanation is correct, it would appear that the *Eustrongylides* infection in *Fundulus* does not give rise to immunity.

TABLE 3.—Distribution of worm cysts within the fish body

Site of cyst	Number of cysts	Percentage of total cysts
Mesentery	602	90.4
Liver	40	6.0
Fat tissue	12	1.8
Peritoneum	9	1.4
Capsule of ovary	3	0.5

A final word may be said about the localization of the parasites within the body of the host. They were found invariably encapsulated in cysts. Most of the cysts occurred along the mesenteries (Table 3). Not a single instance was encountered in which the parasites had settled down within the muscles. This is noteworthy since in other fish species *Eustrongylides* larvae do occur rather regularly in this site (Hunter, 1942; von Brand, 1944).

SUMMARY

1. Both sexes of *Fundulus heteroclitus* are equally parasitized by larval *Eustrongylides ignotus*.
2. The relationships between the size of the fish and the worms seem to indicate a rather slow growth of the latter.
3. Multiple infections are not very rare. Some of them are apparently acquired at the same time, while others indicate the occurrence of superinfection.
4. By far the largest number of worms is found in cysts along the mesenteries.

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STUDIES OF SHEEP PARASITES. V. IMMUNITY TO GASTRO-INTESTINAL NEMATODES*

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Stoll (1929) showed that sheep developed a marked resistance to *Haemonchus contortus* infections, as manifested by a drop in egg count in parasitized animals. Stoll and Nelson (1930) indicated that this resistance was humoral in nature by experiments in which saline extracts of this parasite produced an immediate marked intradermal reaction. Stumberg (1933) by an essentially anaphylactic test was able to detect antibodies of *H. contortus* at a dilution of 1: 50,000. Studies since this time have been confined mainly to a confirmation of Stoll's (1929) work, not only for *H. contortus*, but for the other nematodes parasitic in the alimentary tract of sheep.

Sarles (1937, 1938) developed a new technique for the detection of antibody produced by helminth infections. He showed that when the sera of rats immune to *Nippostrongylus muris* were placed in contact with the preparasitic larvae, a precipitate was formed at various locations on, or in them. These reactions occurred principally about the mouth, excretory pore, cuticle and in the digestive tract. This precipitate decreased the activity of the larvae and inhibited their development. Similar reactions have since been noted by Otto (1940) in *Ancylostoma caninum*, Mauss (1940) and Oliver-González (1940) in *Trichinella spiralis* and Oliver-González (1943) in *Ascaris lumbricoides*.

METHODS

The immune serum was obtained from three lambs and a ewe which had undergone a natural infection and recovery with *H. contortus*, *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Cooperia curticei*, *Nematodirus* sp., *Oesophagostomum columbianum*, *Chabertia ovina* and *Trichuris ovis*. These sheep were kept in the same pasture and maintained under similar conditions. Methods of egg counting have been described by Hawkins et al (1944). The sheep were bled, the blood stored in a refrigerator overnight, and the serum separated the next day.

The larvae were obtained from the feces of lambs with mixed naturally acquired nematode infections. The feces were mixed with charcoal and cultured for two weeks at room temperatures. The larvae were isolated with a Baermann apparatus, and then washed repeatedly in sterile distilled water. They were allowed to stand overnight in sterile distilled water after which they were again washed. The larvae were then placed in 0.1% mercuric chloride for half an hour.

Lapage (1933) and Glaser and Stoll (1940) have shown that solutions of hypochlorite will exsheath strongyle larvae. In order to obtain exsheathed larvae the suspension was placed in 0.2% calcium hypochlorite for half an hour. The larvae were then washed several times in sterile distilled water.

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For the tests the suspensions of larvae and sera were placed on a sterile slide, a cover glass placed on them and ringed with sterile petrolatum. The preparations were kept at 37° C and examined several times a day at both low and high magnification. The species of larvae in each test were not identified.

RESULTS AND DISCUSSION

The egg counts of the ewe and lambs for the summer months are given in Fig. 1. It will be noted that the ewe had a much lower level of parasitism than the lambs. This is characteristic as has been pointed out by Hawkins et al (1944). The resistance of mature sheep to parasitism is much greater than that of the lambs.

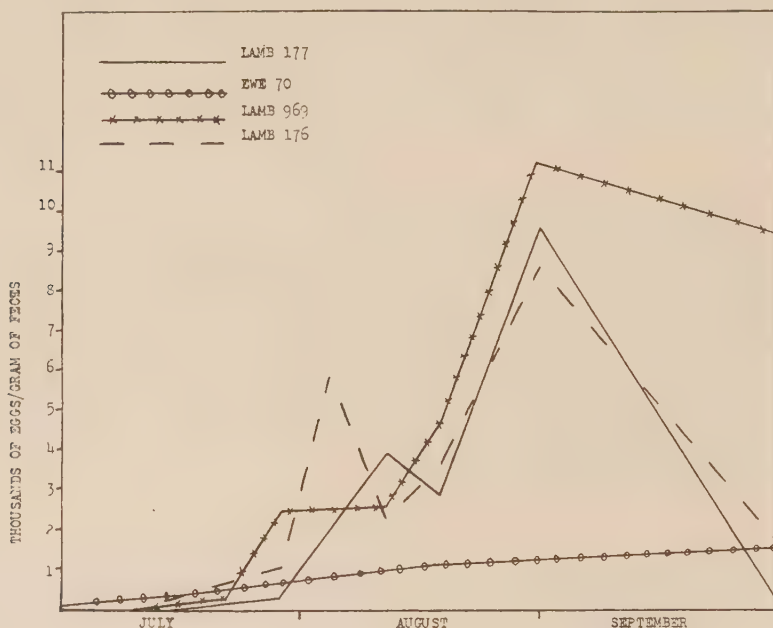


FIG. 1. Total egg counts.

The acquisition of parasitism by the three lambs was similar, although the elimination of the infection or the acquisition of resistance is quite different. Lamb 969 failed to throw off the infection as shown by an egg count of 9600 per gram of feces at the end of the experiment, September 29. Lamb 176 was more successful in ridding itself of parasites, having an egg count of 1800, while lamb 177 lost most of its roundworm parasites as shown by a total egg count of only 300. The effects of this resistance are shown by the weights of these lambs. Lamb 969 was 18% lighter than on July 14; lamb 176 was 4% heavier, and lamb 177 was 6% heavier. While none of these lambs had made economical gains, it was only in those lambs in which a resistance was developing that any weight gains were being made.

In an attempt to determine the nature of this resistance, a suspension of ensheathed nematode larvae was placed in sera of the above-mentioned ewe and lambs. These larvae consisted of *Haemonchus contortus* (68%), *Chabertia ovina* (14%), *Cooperia curticei* (12%), *Oesophagostomum columbianum* (4%) and *Trichostrongylus colubriformis* (2%). No abnormal appearance of the ensheathed larvae could be detected after two weeks' incubation at either 37° C or room temperature.

TABLE I

	Larvae with ppt	Oral ppt	Anal ppt	Excretory pore ppt	Cuticle ppt
	%	%	%	%	%
Ewe 70	78.0	76.0	26.1	18.2	22.0
Lamb 969	45.3	41.5	7.5	0.0	1.8
Lamb 176	48.5	48.5	6.1	0.0	0.0
Lamb 177	88.0	88.0	16.6	9.5	2.3

However, it was noted that a precipitate formed about the mouth of a few of the larvae that had lost their sheaths.

Since it seemed apparent that a precipitate would not form about ensheathed larvae, the sera were next placed in suspensions of exsheathed larvae. In these suspensions a high percentage of the larvae had precipitates about either the mouth, excretory pore, anus or cuticle. The types of oral and anal precipitate are seen in Figs. 2 and 3. The results of this experiment are given in Table I. No precipitates were formed in similar suspensions of larvae placed in the sera of lambs that had been raised parasite free, except for coccidia or *Strongyloides*, or in saline solutions.

FIG. 2. Oral precipitate. $\times 705$.

It may be assumed that this is the type of reaction which enables sheep to throw off the greater part of their parasite burden. After a certain stage in the infection, antibodies are formed. These are capable of forming a precipitate around the mouth and excretory openings of the larvae. Since the severe effects of parasitism in sheep are largely due to the cumulative effects of constant reinfection, it is readily seen that the host may acquire an immunity which will prevent reinfection. Thus, when the larvae are embedded in the gastric or intestinal mucosa, they may be immobilized by this antibody and then the phagocytic cells of the body may destroy them. Such



FIG. 3. Anal precipitate. $\times 705$.

a parallelism between the effects of immune sera *in vitro* and *in vivo* has been demonstrated by Taliaferro and Sarles (1939) in *Nippostrongylus muris* infections and by Oliver-González (1941) in *T. spiralis* infections.

It may be assumed that since lamb 177 had serum capable of forming the precipitate in a large percentage of the larvae *in vitro*, it should have a well-developed immunity. Such was the case as shown by its low egg count and its beginning to gain weight. Serum from lamb 969 formed a precipitate about slightly less than half of the larvae *in vitro*, and as evidenced by its egg count and weight had not developed any marked immunity to reinfection.

It might be presumed from inspection of the egg counts that ewe 70 and lamb 176 would have the same degree of immunity. However, it will be noted that ewe 70 had a relatively low level of infection during the summer, and from inspection of Table I it will be seen that the serum of this ewe, when placed in contact with larvae, produced a precipitate in 78% of the larvae. The ewe had a relatively high degree of immunity all summer. Lamb 176, however, was developing an immunity as indicated by its egg count in Fig. 1. This is shown by the fact that only 48.5% of the larvae placed in the immune serum of this lamb developed a precipitate. This relationship between egg counts at the time serum was obtained and the percentage of larvae reacting with the serum to produce a precipitate is shown in Fig. 4.

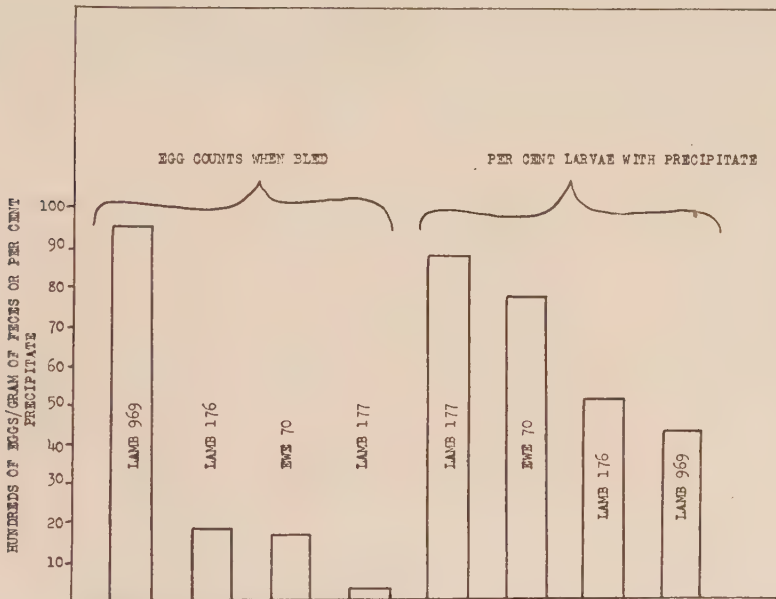


FIG. 4. Relationship between total egg counts and percentage of precipitate formed about larvae.

SUMMARY

Exsheathed strongyle larvae of sheep react to immune serum from naturally infected sheep by the formation of a precipitate in one or more of the following locations: (1) mouth, (2) anus, (3) excretory pore and/or (4) cuticle. The mechanism of immunity against the nematode parasites of the alimentary tract of sheep is postulated.

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PHLEBOTOMUS (DAMPFOMYIA) ANTHOPHORUS, N. SP., AND
PHLEBOTOMUS DIABOLICUS HALL FROM TEXAS
(DIPTERA: PSYCHODIDAE)¹

C. J. ADDIS

During the summer of 1944 a large number of sandflies of the genus *Phlebotomus* was collected at Uvalde, Texas, and brought to Houston in an effort to establish a laboratory colony. Although sandflies had been collected in and around human habitations during the latter part of May and early June, none was found in such localities during July, August or the first part of September. Towards the end of September sandflies were again obtained from human habitations. During the months of July, August and September sandflies were collected from a rabbit pen attached to the Field Experiment Station of the U. S. Bureau of Entomology and Plant Quarantine.

The specimens found in and around homes, biting human beings, were identified as *Phlebotomus diabolicus* Hall, 1936. Those collected from the rabbits proved to be a previously undescribed species possessing a number of unusual characteristics which warrant the erection of a new subgenus to include it, for which the name *Dampfomyia* is proposed. The specific name *anthophorus* is proposed for this new form, based on the peculiar characters of the spermatheca. A description of *Phlebotomus (Dampfomyia) anthophorus*, and an amended description of *Phlebotomus diabolicus* are given below.

Phlebotomus (Dampfomyia) anthophorus, n. sp.

Phlebotomus (Dampfomyia) anthophorus was first observed by Mr. H. M. Brundrett of the Bureau of Entomology and Plant Quarantine in June feeding on rabbits at the Experiment Station in the northern and more elevated part of Uvalde. Observations on these flies were made by the writer at intervals from July to September. The females were found feeding on the rabbits and resting in the vicinity only in the mornings; no males were ever seen. On some mornings up to a hundred flies were collected between 8 and 10 AM, with very few being observed after noon. None were ever seen prior to sunrise or after sundown. The flies ceased to appear late in November, and were not again observed until March 23, 1945.

The rabbit pens where the flies were collected occupied a room attached to the north side of the building and was shaded practically all day. The room was screened on three sides, covered by a roof and provided with a concrete floor. Four or five rabbits were kept separately or in pairs in three large boxes made of wooden slats, with manure pans under the floors. These pans were cleaned only at intervals of several weeks and were thought to be possible breeding places for the flies, but no

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larvae were found, and the absence of any males suggests that the breeding occurred outside the shed.

In order to determine the distribution of this sandfly in Uvalde, observations were made on other rabbits, dogs, cattle, horses, and goats throughout the city, but no *Phlebotomus* was found, even on penned rabbits kept within 50 yards of the Station. Dr. A. Dampf reports (unpublished) collection of a single female specimen, on September 24, 1937, from a house in Cuantla, in the State of Morelos, south of Mexico City.

The flies refused to feed on white rats placed in cages adjoining the rabbits. Attempts to feed them on a human arm in the laboratory at Houston were unsuccessful, but they fed readily on Syrian hamsters. The flies had the characteristic *Phlebotomus* habit of making short hops when disturbed, and were very easily collected.

In trying to locate the breeding places, several caged rabbits were placed by rock piles, old tree stumps, wood rat and ground squirrel nests, goat pens and chicken houses within a radius of a hundred yards of the Station, and in similar locations on the South side of the city. No *Phlebotomus* were found on these rabbits either during the morning or after sundown.

In summary, *P. anthophorus* appears to feed only in the morning, never having been found after sundown. It has a definite preference for rabbit blood but will feed on hamsters in the laboratory. It is apparently not annoying to man since attempts to feed it on human blood have failed, and, except for the single specimen collected by Dampf in Mexico, has not been observed in houses.

Captured females brought back to Houston laid eggs, from which several males and females have developed. A detailed account of the life cycle will be given in a subsequent paper.

Subgenus *Dampfomyia*, new subgenus

Male: Segment V of palpi long, longer than any of the other segments. Geniculate spines of antennae without posterior "spur," extending to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Genitalia with basal segment of upper clasper without tuft of setae. Distal segment with three spines; one apical, one median and ventral, and one on inner side between apical and median spines. Median clasper with setae on distal end, and bearing a large appendage attached to its upper surface and ending distally in a saucer-shaped expansion bearing a number of setae on its margin. Lower clasper longer than median clasper or basal-segment of upper clasper.

Female: Palpi and antennae as in male. Anterior pharynx with lateral teeth on each side; posterior border of floor forming a smooth curve, with a pair of laterally-attached, club-shaped appendages and a chitinized bar on its upper surface. Spermatheca with a lobated, rosette-like expansion around head, followed posteriorly by an enlarged, oval structure which

PLATE I

Phlebotomus (Dampfomyia) anthophorus, n. sp.

- FIG. 1. Antennal segments showing geniculate spines.
- FIG. 2. Palpus.
- FIG. 3. Side view of male genitalia.
- FIG. 4. Head.
- FIG. 5. Anterior pharynx.
- FIG. 6. Spicular apparatus, genital pump and part of spicules.
- FIG. 7. Wing.
- FIG. 8. Side view of female genitalia.
- FIG. 9. Spermatheca (lobated expansion around head somewhat collapsed due to preparation).

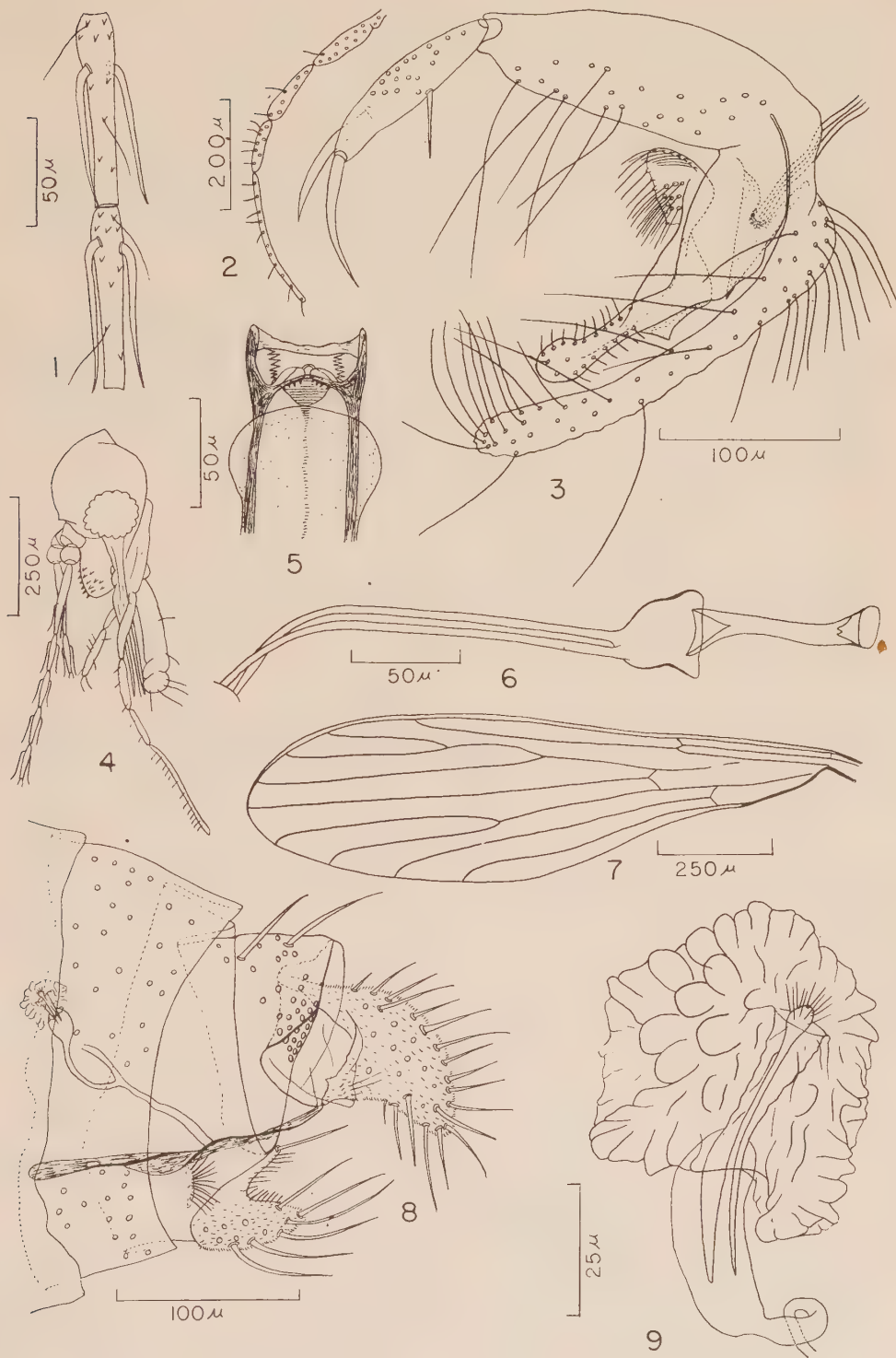


PLATE I

abruptly narrows to form the duct. Ducts fine and membranous, emptying into a narrow common duct.

Larva: Four caudal setae in second to fourth instars.

Phlebotomus anthophorus, n. sp.²

MALE: About 1.3 mm in length; yellowish in color.

Head: Clypeus 116–124 (119) microns in length, longer than the greatest diameter of eyes.

Epipharynx: 177–194 (182) microns in length, measured from anterior border of clypeus.

Palpi: 551–600 (575) microns in length. Measurement of individual segments as follows: I=28–31 (29) microns, II=87–104 (98) microns, III=121–134 (124) microns, IV=83–101 (89) microns, V=219–239 (234) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 3.2 \quad \begin{array}{l} V > II + III; V > III + IV; \\ V < II + III + IV. \end{array}$$

Antennae: Geniculate spines without posterior "spur," extending to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Measurements of individual segments as follows: III=174–198 (185) microns, IV=101–104 (103) microns, V=101–104 (103) microns, VI=97–104 (101) microns, VII=97–101 (99) microns, VIII=94–100 (98) microns, IX=94–97 (96) microns, X=87–97 (93) microns, XI=87–94 (90) microns, XII=83–90 (86) microns, XIII=76–87 (82) microns, XIV=66–80 (73) microns, XV=62–69 (64) microns, XVI=59–62 (60) microns.

$$III < IV + V; III < XII - XVI; IV + V + VI < XII - XVI.$$

$$\frac{III}{\text{epipharynx}} = 1.0 \quad \text{Antennal formula} = \frac{2}{III - XV}$$

Thorax: Mesonotum and scutellum yellowish brown, pleura yellowish.

Wings: Long and narrow, 1220–1317 (1256) microns in length and 343–373 (362) microns in greatest width. Measurements of wing veins as follows: alpha=224–239 (233) microns, beta=209–216 (213) microns, gamma=239–254 (248) microns, delta=52–60 (56) microns.

$$\frac{\text{length}}{\text{width}} = 3.5 \quad \frac{\alpha}{\beta} = 1.1 \quad \frac{\alpha}{\gamma} = 0.94 \quad \frac{\alpha}{\delta} = 4.2$$

Legs: No striking characters.

Forelegs: Femur=561–582 (573) microns, tibia=548–561 (556) microns, tarsal segments: I=287–313 (303) microns, II=168–179 (171) microns, III=104–122 (118) microns, IV=89–104 (101) microns, V=60–75 (70) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 0.97 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Midlegs: Femur=582–604 (592) microns, tibia=686–722 (702) microns, tarsal segments: I=358–373 (365) microns, II=179–194 (187) microns, III=119–134 (127) microns, IV=96–104 (101) microns, V=75–81 (79) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0$$

Hindlegs: Femur=627–658 (639) microns, tibia=1000–1114 (1028) microns, tarsal segments: I=432–454 (441) microns, II=224–243 (233) microns, III=134–152 (143) microns, IV=104–121 (112) microns, V=75–89 (82) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.6 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.9$$

² Measurements based on a study of six flies, average given in parenthesis.

PLATE II

Phlebotomus diabolicus Hall

- FIG. 10. Spicular apparatus, genital pump and part of spicules.
- FIG. 11. Palpus.
- FIG. 12. Side view of male genitalia.
- FIG. 13. Antennal segments showing geniculate spines.
- FIG. 14. Anterior pharynx.
- FIG. 15. Wing.
- FIG. 16. Side view of female genitalia.
- FIG. 17. Spermatheca.
- FIG. 18. Head.

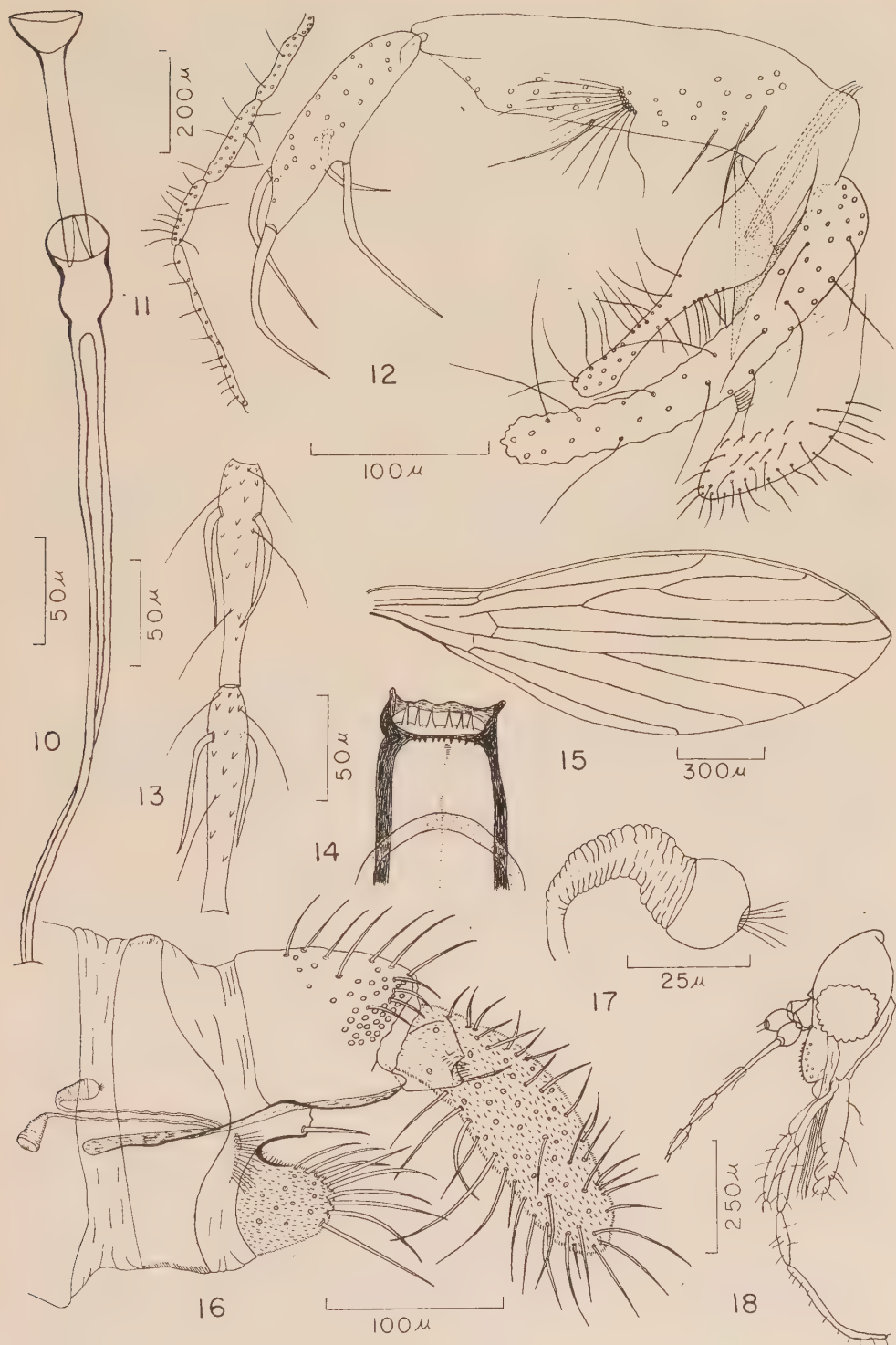


PLATE II

Abdomen: No striking characters.

Genitalia (Plate I, Fig. 3): Basal segment of upper clasper 179–209 (191) microns in length, with no tuft of setae. Distal segment 118–125 (120) microns in length, with three spines arranged in following manner: one apical; one median and ventral, finer than apical one; and one, similar to apical spine, inserted on inner side midway between apical and median spines.

Median clasper: 149–198 (174) microns in length, with fine setae and with an inner appendage, attached along middle third of dorsal side of clasper, and terminating in a saucer-shaped expansion with about 25 setae arranged around margin.

Lower clasper: 254–274 (259) microns in length, definitely longer than median clasper or basal segment of upper clasper.

Spicular apparatus (Plate I, Fig. 6): genital pump 101–111 (105) microns in length, spicules 288–303 (294) microns in length.

FEMALE: Larger than male (about 2 mm in length); coloration as in male.

Head (Plate I, Fig. 4): Similar to male, clypeus 125–149 (141) microns in length.

Epipharynx: 209–239 (225) microns in length, measured from anterior border of clypeus.

Palpi (Plate I, Fig. 2): 524–591 (570) microns in length. Measurements of individual segments as follows: I=31–35 (34) microns, II=83–104 (95) microns, III=115–132 (127) microns, IV=83–101 (94) microns, V=208–232 (220) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 2.5 \quad \begin{array}{l} V \pm II + III; V \pm III + IV; \\ V < II + III + IV. \end{array}$$

Antennae (Plate I, Fig. 1): Geniculate spines as in male. Measurements of antennal segments as follows: III=146–180 (163) microns, IV=73–87 (79) microns, V=73–87 (79) microns, VI=73–87 (79) microns, VII=73–87 (79) microns, VIII=73–87 (79) microns, IX=73–85 (78) microns, X=73–83 (78) microns, XI=73–80 (77) microns, XII=69–76 (73) microns, XIII=66–73 (69) microns, XIV=52–62 (59) microns, XV=45–52 (49) microns, XVI=38–49 (45) microns.

$$\begin{array}{l} III > IV + V; III < IV + V + VI; III < XII - XVI; \\ IV + V + VI < XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.72 \quad \text{antennal formula} = \frac{2}{III - XV}$$

Anterior pharynx (Plate I, Fig. 5): No horizontal teeth but with about seven lateral teeth on each side. Posterior border of floor forming a smooth curve, and with a pair of laterally attached, club-shaped appendages and a chitinized bar on its upper surface. Vertical teeth few (about 8 in number) and small. Pigmented area triangular in shape and very rough at posterior end, followed by a long and narrow area.

Posterior pharynx: No striking characters.

Thorax: As in male.

Wings (Plate I, Fig. 7): 1463–1524 (1494) microns in length and 433–507 (459) microns in greatest width. Wing veins with measurements as follows: alpha=298–373 (333) microns, beta=224–269 (242) microns, gamma=269–298 (291) microns, delta=63–149 (116) microns.

$$\frac{\text{length}}{\text{width}} = 3.3 \quad \frac{\alpha}{\beta} = 1.4 \quad \frac{\alpha}{\gamma} = 1.1 \quad \frac{\alpha}{\delta} = 2.0$$

Legs: No striking characters.

Forelegs: Femur=522–597 (545) microns, tibia=494–522 (515) microns, tarsal segments: I=283–328 (301) microns, II=164–179 (171) microns, III=104–119 (117) microns, IV=90–104 (92) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 0.94 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Midlegs: Femur=537–627 (567) microns, tibia=598–656 (622) microns, tarsal segments: I=328–388 (351) microns, II=179–224 (194) microns, III=119–149 (134) microns, IV=90–119 (104) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.1 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Hindlegs: femur=612–686 (642) microns, tibia=821–910 (863) microns, tarsal segments: I=462–492 (473) microns, II=209–239 (229) microns, III=134–149 (144) microns, IV=104–119 (112) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.3 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.1$$

Abdomen: with erect bristles.

Spermatheca (Plate I, Figs. 8 and 9): With a lobated, rosette-shaped expansion around head; head small with fine setae, followed posteriorly by an enlarged oval structure which narrows abruptly to form the duct. Ducts fine and with smooth membranous walls, emptying into a narrow common duct.

Type material. *Holotype*: Male (stained whole mount); reared in the biological laboratories of the Rice Institute by C. J. Addis during the summer of 1944 from eggs laid by females collected in Uvalde, Texas, July, 1944. To be deposited in the United States National Museum.

Allotype: Female (stained dissected mount); collected by C. J. Addis at Uvalde, Texas, July, 1944. To be deposited in the United States National Museum.

Paratypes: Several females collected with the allotype and several males reared in the laboratory with the holotype. To be deposited in the collections of Asa C. Chandler and C. J. Addis.

Taxonomic discussion: See below *Phlebotomus diabolicus*.

Phlebotomus diabolicus Hall, 1936

Hall (1936) described *Phlebotomus diabolicus* from specimens captured at Uvalde, and Lindquist (1936) published notes on its habits and biology. The observations of the writer corroborate those of Hall and Lindquist. During the past season specimens of *P. diabolicus* were first noticed in the houses at night during the latter part of May. They disappeared toward the middle of June and did not reappear until the last of September. During October and up to November 16, when the writer received the last shipment of specimens from Uvalde, the flies could be caught in fair numbers at night under the lights in houses and on lawns. Females shipped to the laboratory in Houston readily fed on a human arm, and some took blood meals from Syrian hamsters. Eggs laid in the laboratory failed to develop beyond the fourth larval stage.

Male: 2 mm in length; blackish-brown in color with silvery sheen.

Head: Clypeus 142–149 (146) microns in length, shorter than greatest diameter of eyes.

Epipharynx: 240–264 (255) microns in length, measured from anterior border of clypeus.

Palpi: 634–721 (673) microns in length. Measurements of individual segments as follows: I = 28–35 (31) microns, II = 121–142 (131) microns, III = 153–173 (163) microns, IV = 104–125 (113) microns, V = 232–246 (240) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 2.6 \quad \begin{array}{l} V < II + III; V < III + IV; \\ V < II + III + IV. \end{array}$$

Antennae: geniculate spines without posterior "spur," extending about three-fourths the distance to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Measurements of individual segments as follows: III = 222–249 (237) microns, IV = 101–118 (108) microns, V = 101–118 (108) microns, VI = 97–115 (106) microns, VII = 97–115 (104) microns, VIII = 94–111 (101) microns, IX = 94–101 (97) microns, X = 90–97 (95) microns, XI = 87–94 (92) microns, XII = 83–90 (86) microns, XIII = 76–83 (79) microns, XIV = 59–66 (63) microns, XV = 56–59 (57) microns, XVI = 49–52 (51) microns.

$$\begin{array}{l} III > IV + V; III > IV + V + VI; III > XII - XVI; \\ IV + V + VI > XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.93 \quad \text{antennal formula} = \frac{2}{III - XV}$$

Thorax: Mesonotum and scutellum blackish-brown, pleura lighter.

Wings: Bluntly lanceolate, 1854–1902 (1872) microns in length and 627–642 (634) microns in greatest width. Measurements of wing veins as follows: alpha = 448–470 (459) microns, beta = 245–283 (265) microns, gamma = 358–388 (367) microns, delta = 104–134 (112) microns.

$$\frac{\text{length}}{\text{width}} = 3.0 \quad \frac{\alpha}{\beta} = 1.7 \quad \frac{\alpha}{\gamma} = 1.3 \quad \frac{\alpha}{\delta} = 4.1$$

Legs: no striking characters.

Forelegs: Femur = 716–731 (724) microns, tibia = 731–754 (742) microns, tarsal segments: I = 373–418 (398) microns, II = 209–239 (226) microns, III = 130–149 (138) microns, IV = 119–134 (125) microns, V = 75–90 (86) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.0 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Midlegs: Femur = 731–761 (748) microns, tibia = 878–910 (888) microns, tarsal segments: I = 442–467 (453) microns, II = 239–254 (245) microns, III = 138–164 (153) microns, IV = 119–128 (121) microns, V = 86–93 (89) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Hindlegs: Femur = 791–836 (819) microns, tibia = 1119–1149 (1135) microns, tarsal segments: I = 566–612 (592) microns, II = 283–303 (291) microns, III = 179–194 (183) microns, IV = 134–149 (145) microns, V = 90–104 (95) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.4 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0$$

Abdomen: No striking characters.

Genitalia (Plate II, Fig. 12): Basal segment of upper clasper 194–236 (213) microns in length, with a well-defined tuft of setae on inner side. Distal segment 148–163 (156) microns in length, with four long spines arranged in the following manner: one apical; one median and ventral; one median on inner side; and one dorsal, midway between apical and median spines.

Median clasper: 164–184 (177) microns in length.

Lower clasper: 205–246 (233) microns in length, longer than median clasper or basal segment of upper clasper.

Spicular apparatus (Plate II, Fig. 10): Genital pump 135–153 (142) microns in length, spicules 388–412 (399) microns in length.

FEMALE: 2 mm in length, coloration as in male.

Head (Plate II, Fig. 18): Similar to male, clypeus 146–153 (151) microns in length.

Epipharynx: 269–298 (281) microns in length, measured from anterior border of clypeus.

Palpi (Plate II, Fig. 11): 792–865 (838) microns in length. Measurements of individual segments as follows: I = 31–42 (38) microns, II = 139–156 (149) microns, III = 167–184 (179) microns, IV = 118–139 (132) microns, V = 330–347 (339) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 3.0 \quad \begin{array}{l} V > II + III; V > III + IV; \\ V < II + III + IV. \end{array}$$

Antennae (Plate II, Fig. 13): Geniculate spines as in male. Measurements of antennal segments as follows: III = 226–243 (235) microns, IV = 101–104 (103) microns, V = 101–104 (103) microns, VI = 97–104 (101) microns, VII = 97–101 (99) microns, VIII = 94–101 (97) microns, IX = 90–101 (96) microns, X = 87–97 (93) microns, XI = 79–94 (89) microns, XII = 76–90 (86) microns, XIII = 73–87 (80) microns, XIV = 69–76 (73) microns, XV = 59–69 (64) microns, XVI = 59–62 (60) microns.

$$\begin{array}{l} III > IV + V; III < IV + V + VI; III < XII - XVI; \\ IV + V + VI < XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.83 \quad \text{antennal formula} = \frac{2}{III - XV}$$

Anterior pharynx (Plate II, Fig. 14): With six horizontal teeth. Posterior border of floor forming a smooth curve, with a chitinated bar on its upper surface. Vertical teeth few and small. Pigmented area long and narrow.

Posterior pharynx: No striking characters.

Thorax: As in male.

Wings (Plate II, Fig. 15): 1805–2000 (1927) microns in length and 627–686 (669) microns in greatest width. Measurements of wing veins as follows: alpha = 537–582 (565) microns, beta = 254–269 (259) microns, gamma = 336–343 (341) microns, delta = 164–179 (172) microns.

$$\frac{\text{length}}{\text{width}} = 2.9 \quad \frac{\alpha}{\beta} = 2.2 \quad \frac{\alpha}{\gamma} = 1.7 \quad \frac{\alpha}{\delta} = 3.3$$

Legs: No striking characters.

Forelegs: Femur = 716–746 (731) microns, tibia = 776–806 (791) microns, tarsal segments: I = 388–418 (400) microns, II = 224–242 (236) microns, III = 145–149 (147) microns, IV = 119–129 (123) microns, V = 90–93 (92) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.1 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.7$$

Midlegs: Femur = 746–776 (762) microns, tibia = 880–910 (890) microns, tarsal segments: I = 448–477 (467) microns, II = 239–254 (247) microns, III = 164–179 (168) microns, IV = 134–143 (137) microns, V = 90–93 (92) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.9$$

Hindlegs: Femur = 836–880 (859) microns, tibia = 1179–1268 (1209) microns, tarsal segments: I = 612–642 (628) microns, II = 298–328 (311) microns, III = 169–194 (179) microns, IV = 149–164 (154) microns, V = 90–104 (95) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0$$

Abdomen: With erect bristles.

Spermatheca (Plate II, Figs. 16 and 17): With smooth globular head, followed posteriorly by a rugose area. Ducts fine, with smooth membranous walls, and without a common duct.

Material examined: Six males and six females from Uvalde, Texas, collected by Mr. and Mrs. H. M. Brundrett, October and November, 1944.

This material was compared with the female allotype captured by A. W. Lindquist at Uvalde, Texas, November 14, 1934, and also with the description and drawing of a male by Hall (1936), and found to be specifically identical.

Taxonomic discussion: The addition of *Phlebotomus* (*Dampfomyia*) *anthophorus* makes a total of six known species of *Phlebotomus* in the United States: *P. vexator* Coquillett, which has been taken throughout the southern states, from Maryland to California; *P. diabolicus* Hall, from Texas; *P. texanus* Dampf, from Texas; *P. stewarti* Mangabeira and Galindo, from California; *P. limai* Fonseca, with a wide distribution in southern United States, having been taken in Alabama, Mississippi and North Carolina (Rozeboom, 1944); and *P. anthophorus*, from Texas. Specimens of *Phlebotomus* have been collected in Florida also, but have not been specifically identified.

P. anthophorus can readily be distinguished from the other known species of *Phlebotomus* in the United States by the appendage on the median clasper of the male genitalia, the peculiar characters of the anterior pharynx, and the flower-like spermathecae of the female.

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A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *POLYMORPHUS* FROM THE AMERICAN COOT

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For many years the writer has been accumulating specimens of an unidentified acanthocephalan from the American coot (*Fulica americana*). Most of the specimens have been obtained as the result of routine field investigations of food habits of the birds. Until very recently all but two of the individuals have lacked the proboscis, since that organ had been broken off upon removal of the worms from the host intestine. The two complete individuals, both females, were collected by Dr. Justus F. Mueller from a coot on Oneida Lake in New York state. The proboscis on each of these two individuals was so distinctively different from that known for any other species that the slides were put aside in the belief that the shapes might be due to some procedure in handling or in killing the parasites. In November of 1944, the writer received from Mr. Robert Rausch a consignment of unidentified ACANTHOCEPHALA. Among these were specimens from six individuals of *Fulica americana* taken on Buckeye Lake in Ohio on November 11, 1943. Many of these specimens had been carefully dissected out from the host intestine and of these all of the females bore an inflated proboscis, of pear-like shape, specifically unlike that of any described species but identical with the two individuals from Oneida Lake. The enlarged, bulbous proboscis explains the readiness with which the anchored proboscis becomes broken from the neck when an attempt is made to dislodge the female worms from the host intestine. A new species of *Polymorphus* recognized for these forms is here described under the name *Polymorphus trochus*.

Polymorphus trochus n. sp.

(Figs. 1-5)

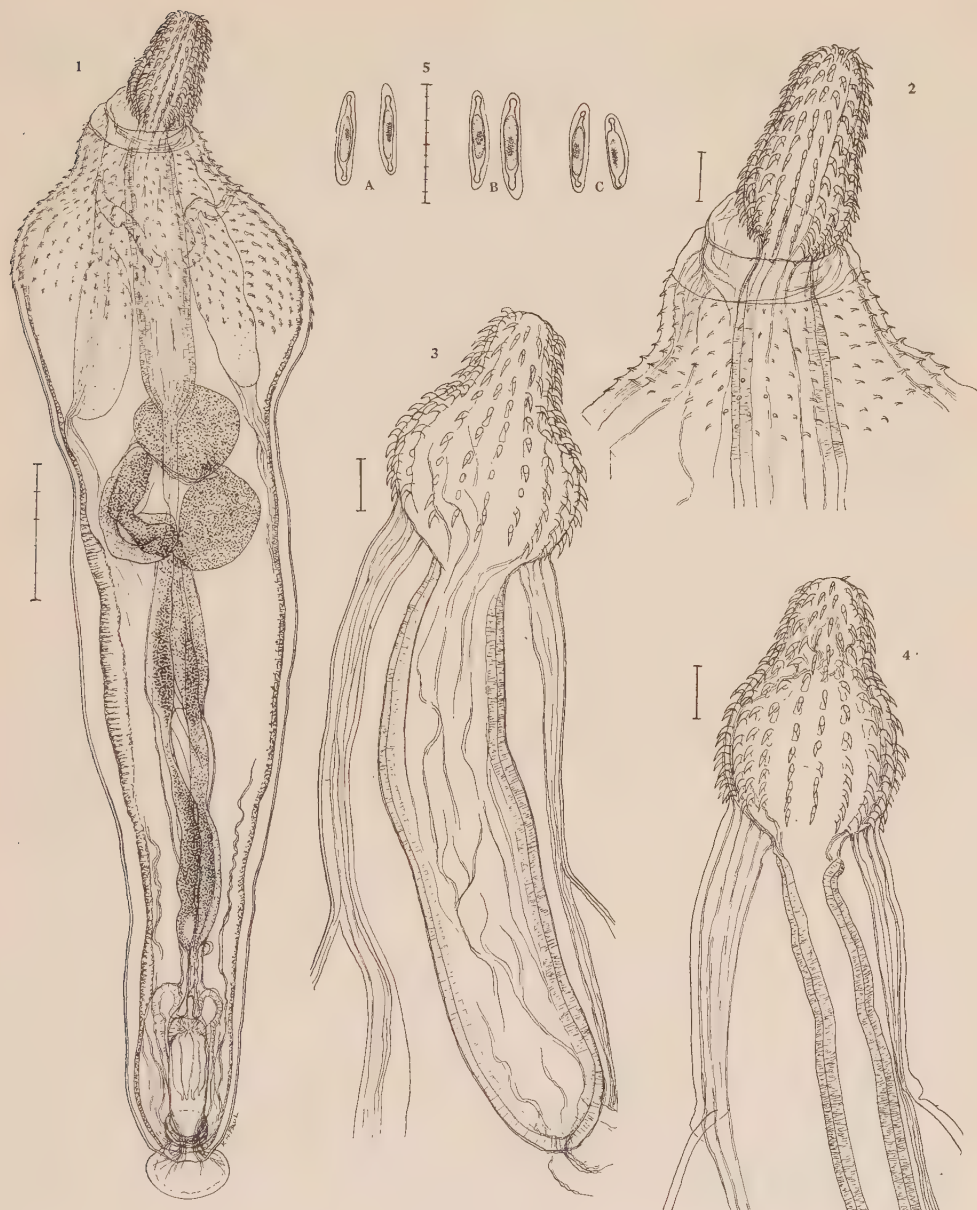
With the characteristics of the genus *Polymorphus*, modified from Meyer (1932) to include his genus *Profilicollis*. Body of females from 4.5 to 7.8 mm long; males, about 3.8 mm. Greatest diameter slightly back of the front end of the body-proper, gradually tapering posteriorly to a narrowed posterior extremity and anteriorly rounding rapidly to meet a relatively narrow neck. Swollen trunk region set with numerous cuticular spines. The neck, spineless, sub-cylindrical to barrel-shaped, slightly reduced in diameter at both ends, is from 0.4 to 1.1 mm long and from 0.35 to 0.54 mm in maximum diameter. Proboscis in female pear-shaped, much inflated at base, with a short, greatly reduced nipple-like termination. Proboscis of females about 0.38 to 0.56 mm long; from 0.30 to 0.49 mm in maximum diameter with reduced terminal portion 0.100 to 0.175 mm in diameter. Proboscis of observed males, without conspicuous inflation, about 0.385 to 0.560 mm long; 0.210 mm in maximum diameter and 0.140 mm in terminal portion. Most of the hooks near the middle zone of the proboscis about 35 μ long, many at both ends somewhat shorter (near anterior extremity 21 to 42 μ long; on basal region 21 to 35 μ).

Embryos within body of mature females 75 to 84 μ by 14 to 20 μ .

Host: In intestine of American coot (*Fulica americana*) of Buckeye Lake, Ohio; Oneida Lake, New York; and Illinois River in Illinois.

Holotype: Female No. VC 3787.2; allotype male No. VC 3789.2 and series of paratypes (13 females and 1 male) in collection of H. J. Van Cleave, Urbana, Illinois.

In the past the line of demarcation between the genera *Polymorphus* and *Filicollis* has been misinterpreted often. Species now thought of as belonging to



EXPLANATION OF PLATE
Polymorphus trochus new species

All drawings were made with a camera lucida from stained permanent mounts in Damar. The writer is indebted to Mrs. Katharine Hill Paul for preparation of the drawings. The scale indicating magnification beside Fig. 1 has the value of 0.5 mm; all other scales on this plate have the value of 0.1 mm.

FIG. 1. Drawing of entire male (allotype VC 3789.2). The four cement glands are somewhat twisted and contorted in the region to the left of the testes. Note the two retinacula emerging from the wall of the proboscis receptacle anterior to the middle of that organ.

FIG. 2. Details of proboscis and anterior region of trunk in allotype male. Note that neck region is partially retracted within the body.

FIG. 3. Anterior extremity of a paratype female, showing the characteristic proboscis of the female of this species and its relation to the neck and receptacle of the proboscis.

FIG. 4. Proboscis and neck of holotype female (VC 3787.2).

FIG. 5. Three groups of embryos from different parts of the body cavity of a gravid female: A, from neck region; B, from middle of body; C, from near posterior extremity.

one of these genera have been mistakenly ascribed to the other because the importance of certain characteristics has been underestimated or ignored. At times this has resulted in the attempt to create new intermediate genera or to emend the generic diagnoses so as to include the intermediate forms in one of the genera already recognized. Thus in 1916 the writer suggested (page 132) that the inflated form of the proboscis, which some earlier workers had presumably shown to be a character appearing late in ontogeny, might be omitted from the list of diagnostic characters for the genus *Filicollis*. By a series of gradual steps in the literature, the species originally described as *Filicollis botulus* and *F. arcticus* were eliminated from *Filicollis* (see Meyer, 1931, Witenberg, 1932, and Van Cleave, 1939) and came to rest in the genus *Polymorphus*. This line of action took place in spite of the fact that at least several research workers had come to the conclusion that form of the inflated proboscis, regardless of the pattern of distribution of the proboscis hooks, was a character restricted to the genus *Filicollis* among the genera of avian POLYMORPHINAE.

Consequently, when the new species described in this paper as *P. trochus* was first studied, the swollen proboscis of the female seemed to call for assignment to *Filicollis*.

More recently, the writer has been restudying the problem of generic characters for the separation of the genera *Filicollis* and *Polymorphus* and has come to the conclusion that the distinctive arrangement and limitation of the hooks to the anterior face of the inflated, spheroidal proboscis is likewise distinctive of the genus *Filicollis*. These features of hook arrangement were so diverse from those found on the genotype, *Filicollis anatis*, that other features such as the form of the neck gave support to the proposal for including the species *trochus* within the genus *Polymorphus*.

P. trochus differs from all other species assigned to *Polymorphus* in the distinctive form of the proboscis. Though the proboscides of females of *P. botulus* and *P. arcticus*, are inflated and spheroidal at the end of an attenuated neck, the inflated proboscis of the female of *P. trochus* carries a terminal portion much reduced in size, projecting nipple-like from the inflated zone.

The very small numbers of males in the type material of *P. trochus* remains unexplained. The relatively smaller size of the male proboscis, lacking the bulbular enlargement, would be a factor predisposing toward recovery of a greater percentage of entire males than of females from the host intestine. Yet in the available collections there are 16 individuals with body and proboscis perfect enough to leave no doubt as to specific identity. Among these 16 individuals, all but two are female.

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STUDIES ON THE LIFE HISTORY OF *BRACHYLECITHUM AMERICANUM* N. SP., A LIVER FLUKE OF PASSERINE BIRDS¹

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Between 1938 and 1941 attempts were made to determine experimentally the life cycles of the common microcoeliid trematodes occurring in hosts in the vicinity of Houston, Texas. The results of the first of these studies were given in a recent paper (Denton, 1944) in which the morphology and development of the larval stages of *Eurytrema procyonis* Denton, 1942, were described. The present paper, the second of the series, gives in detail the results previously reported in an abstract (Denton, 1941) of experimental studies on the life history of *Brachylecithum americanum* n. sp. In this investigation, which was conducted simultaneously with the one on *E. procyonis*, the same techniques of handling and infecting snails and the same methods of study were employed (for methods, see Denton, 1944). For experimental infections, laboratory-bred snails were used throughout.

BIOLOGY AND LIFE HISTORY

The adult *B. americanum* is a common parasite in the biliary ducts of the livers of certain birds of the families Icteridae and Corvidae. In a series of birds from Georgia, Tennessee, and Texas, 65 per cent of the meadowlarks, 50 per cent of the mesquite grackles, 18 per cent of the bronzed grackles, and 22 per cent of the crows examined harbored this trematode. One of 12 blue jays examined contained immature forms which were assigned to this species. Individual birds have been found infected with from a few to more than 100 specimens of this worm.

Since adults of *B. americanum* could not be induced to discharge their mature eggs in either physiological saline or tap water, and since the avian hosts of this worm were found harboring other microcoeliids whose eggs are indistinguishable from those of this species, only eggs teased from the uteri of worms were used in the feeding experiments. Worms from meadowlarks collected in the vicinity of Houston, Texas, supplied the eggs employed in this study. The eggs, after incubation at 38–39° C for 48 hours, were mixed with a little cornmeal and fed to the following terrestrial snails: *Polygyra texasiana* (Moricand), *Stenotrema fraterna aliciae* (Pilsbry), *Meşodon thyroidus* (Say), *Praticollega berlandieriana* (Moricand), *Anguispira alternata* (Say), *Bulimulus alternatus mariae* (Albers), and *Deroceras agrestis* (Linnaeus). The eggs hatched in all seven species of snails but development proceeded only in *P. texasiana* and *P. berlandieriana*. As well as could be determined, all mature eggs had hatched within three hours, the hatching occurring in the upper small intestine of the host. The miracidia apparently penetrated the intestinal wall in this region and passed to the middle digestive gland where they continued their development.

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¹ A contribution from the Department of Biology, The Rice Institute, Houston, Texas.

² The writer expresses his appreciation to Professor Asa C. Chandler under whose direction this investigation was done, to Dr. Paul Bartsch of the U. S. National Museum for identification of the snail hosts, and to Dr. H. J. Reinhard of the Texas Agricultural Experiment Station for the identification of certain insects.

In the first experiment, *B. americanum* eggs were fed to 13 adult *P. texasiana*, of which six developed heavy infections, one failed to become infected, one examined on the 35th day after exposure may have been infected, although no sporocysts were detected, and five died and decomposed before examination. The experiment was repeated using 20 adult snails, of which 13 developed infections and seven died and disintegrated. In the first attempt to infect *P. berlandieriana*, eggs were fed to 20 very young snails, 15 of which died and decomposed before examination; the remaining 5 developed heavy infections. On repetition of this experiment, using 15 adult snails, 11 developed heavy infections, two died and were not examined, and two examined on the 35th day after exposure may have contained infections that were not detected.

In the laboratory the rates of development in the two molluscan hosts were almost identical. Mother sporocysts in both hosts were first detected with the dissecting microscope on the 50th day of infection. Three snails examined at this time contained 5, 8, and 10 mother sporocysts respectively. With the exception of one organism situated just beneath the epithelium, all the sporocysts were located in the haemocoelic space deep between the lobes of the middle digestive gland. The mothers already contained recognizable daughter sporocysts. Counts of the daughter embryos developing in different mothers ranged from 50 to 70. All of the daughter sporocysts were at approximately the same stage of development.

The mother sporocysts, when again studied on the 64th day, had increased considerably in size. In five snails examined at this time, the mothers were greatly distended by the enclosed daughter sporocysts. Each comprised a mass of daughters which almost completely filled the body cavity, leaving very little matrix. The daughters were at the migrating stage and seemed ready to emerge from the parents.

Some time between the 64th and 70th day of infection the mother sporocysts ruptured and liberated their offspring in the haemocoelic space of the hosts. In a snail examined on the 70th day of infection and in all snails examined thereafter the mother sporocysts had ruptured. Apparently, the rupturing of the mother sporocyst results from continuous stretching caused by the growth of the enclosed daughters. After rupturing, the remains of the mother sporocysts seemed to be absorbed, since no evidence of them was found in snails examined on later dates. There was no evidence that daughter sporocysts might sometimes complete their development without emerging from the parent form.

When the migrating daughter sporocysts were placed in 0.5 per cent saline, they were very active and capable of extending to three times their contracted length. The anterior "neck" was especially mobile and showed lateral movements suggestive of those of nematodes.

After liberation from the parent form, the daughter sporocysts became dispersed in the haemocoelic space throughout the body of the snail. In a snail dissected on the 70th day of infection, sporocysts were most numerous in the digestive gland but were present also in connective tissue along the intestine, in the albumen gland, in the wall of the kidney, and in the respiratory portion of the mantle. In all snails examined thereafter, sporocysts were found throughout the soft tissues of the body. Some were present even in the dorsal part of the foot. The preferred site for development seemed to be the branches of the pulmonary vein, but because of the heavy experimental infections (the snails contained 3,000 to 4,000 sporocysts) it was impossible for all of them to crowd into this location.

By the 100th day of infection the daughter sporocysts contained some fully formed cercariae along with germ balls in various stages of development. However, it was not until the 106th day that cercariae were expelled in the culture dishes from infected *P. texasiana*, and the 109th day from *P. berlandieriana*. The cercariae escaped from the daughter sporocysts through the cervical birth canal and passed into the mantle cavity where they collected in compact masses of from 150 to 300, held together by a slimy, viscid material secreted from their large glands. These cercarial masses were then expelled from the respiratory pore and were deposited on vegetation and other objects as the snails crawled about. Occasionally, a few cercariae were expelled mixed with the feces.

In the laboratory, individual snails expelled cercarial masses almost every day. Usually the masses were expelled during the night though some were expelled late in the afternoon. Just what factors controlled the escape of the cercariae into the mantle cavity, and then their expulsion in slimy masses were not determined. It is probable that in nature such factors as sunshine, and dry and rainy weather, which greatly affect the snails, in turn exert an influence on the cercariae.

Masses of cercariae were observed at different times and under various conditions after being expelled in the culture dishes and on leaves of several plants. Under no conditions were either group or individual cysts formed. When placed in water or 0.5 per cent saline the cercarial masses immediately dissolved in the liquid. In the culture dishes they dried up in a short time unless the atmosphere was kept saturated. However, cercarial masses deposited on transpiring leaves remained moist until the death of the cercariae.

The number of cercariae which developed in individual snails was enormous. Two heavily infected *P. texasiana* expelled from one to five cercarial masses daily for over a month and were still expelling them at a slower rate after three months. Each of these snails is conservatively estimated to have produced well over 20,000 cercariae.

Freshly expelled cercariae slowly extended and contracted within the viscid mass. When the mass was dissolved in water or 0.5 per cent saline, the cercariae immediately became very active. The body was extended until it was long and narrow, then rapidly lashed about, bending laterally and dorso-ventrally. At the same time the tail was slowly extended, then suddenly snapped ventrally in a tight coil (Fig. 5), the cercariae apparently trying actively to bore into or cling to something. After about 30 minutes of this intense activity, however, they were almost spent. They became sluggish and inched slowly over the bottom of the container, and in 6 to 8 hours were dead. Cercariae left undisturbed in masses deposited on leaves of plants remained alive for 24 to 36 hours. After the cercariae died, the mass liquified and disintegrated.

Although *Dicrocoelium dendriticum* (Rudolphi, 1819), the only member of the subfamily DICROCOELIINAE LOOSS, 1899, for which the complete life cycle has been worked out, has been shown to be capable of infecting the definitive host without utilizing a second intermediate host, there is some reason to believe that this is not a uniform condition in the subfamily, and may, indeed, be exceptional. In this form the cercariae become enclosed in slime balls which are more or less hardened peripherally, forming resistant, cyst-like structures.

In the closely related genus *Eurytrema*, according to observations made by the writer (Denton, 1944) the cercariae do not escape from the daughter sporocysts

at all, but are contained in "endocysts" inside the parent, the body wall of the latter forming an additional protective covering. Whether or not these forms require second intermediate hosts has not been determined, but it is possible that they do, since attempts to infect the definitive hosts (crow and blue jay) of *Eurytrema* sp. by feeding sporocysts that had recently escaped from a snail were unsuccessful (unpublished data).

In some of the other dicrocoeliids of birds, including *B. americanum* here under consideration, there is reason to believe that a second intermediate host might be required. Several unsuccessful attempts were made to infect definitive hosts (blue jays) with *Lutztrema* sp. both by feeding expelled cercariae and by feeding infected snails (unpublished work). It was also observed that all cercariae of this species fed to birds had disappeared from the mouth and oesophagus, and were digested in the stomach, within 30 minutes after feeding. Furthermore, it seemed improbable that the cercariae of *Brachylecithum* and *Lutztrema*, unprotected by cyst-like structures of any kind, were destined to be picked up and swallowed by their definitive hosts, which are predominantly insect-eating birds.

On the basis of these considerations, attempts were made to find a second intermediate host. Careful dissections of a number of snails with mature infections showed that encystment does not occur in snails with primary infections. Furthermore, exposure of uninfected snails of several species to snails expelling cercariae showed that no transfer of cercariae between individuals with subsequent encystment occurs. It was evident that the encystment host, if one is utilized, must be sought for among the other invertebrates eaten by birds.

Earthworms and many types of arthropods which conceivably might serve as encystment hosts were tested. These invertebrates were exposed to infection in various ways. Some were placed for various lengths of time in the cultures with snails expelling cercariae; some were fed cercariae on lettuce and other plant leaves; and some were exposed by smearing cercariae on the surface of their bodies. These attempts were without success until 10 dicrocoeliid metacercariae, morphologically indistinguishable from those of *B. americanum*, were recovered from a 12-spotted cucumber beetle, *Diabrotica duodecimpunctata*, which had been exposed to the infected snails for two days. Three of the metacercariae from this beetle were enclosed in clear hyaline cysts, while the others were free in the fat bodies. The fact that several of the nonencysted metacercariae seemed to be partly chitinized suggested a previous natural infection. Whether the living metacercariae were acquired from the experimental infection or naturally, there could be no doubt of their belonging to this species or one very closely related to it.

Following this clue to the probable group of insects involved, seven adults and six larvae of the chrysomelid beetle, *Gastroidia cyanea*, were exposed to cercariae on dock (*Rumex* sp.) leaves given them for food. When examined from one to five days later, the larvae contained from one to six nonencysted metacercariae in their abdominal cavities. These were the same in every respect as the *B. americanum* cercariae to which they had been exposed, except for the lack of the tails. The adult beetles contained no cercariae. Whether the cercariae entered the larvae by mouth or by some other route was not determined. While it is possible that the beetle larvae had acquired natural infections prior to collection, the chance of all six larvae having acquired such infections just prior to the experimental exposure,

which would have to be postulated since the metacercariae were still free in the body cavity, seems too remote to be given very serious consideration.

The above observations, though not conclusive, suggest that chrysomelid beetles serve as the natural second intermediate host of this worm, and that birds become infected from eating beetle larvae containing encysted metacercariae or adult beetles which acquired metacercariae during their larval stages. This hypothesis could not be tested at the time because of insufficient material, and further work on the problem by the writer has not since been feasible. The observations made are recorded in the hope that although incomplete, they may serve as a guide to further work on this group of flukes.

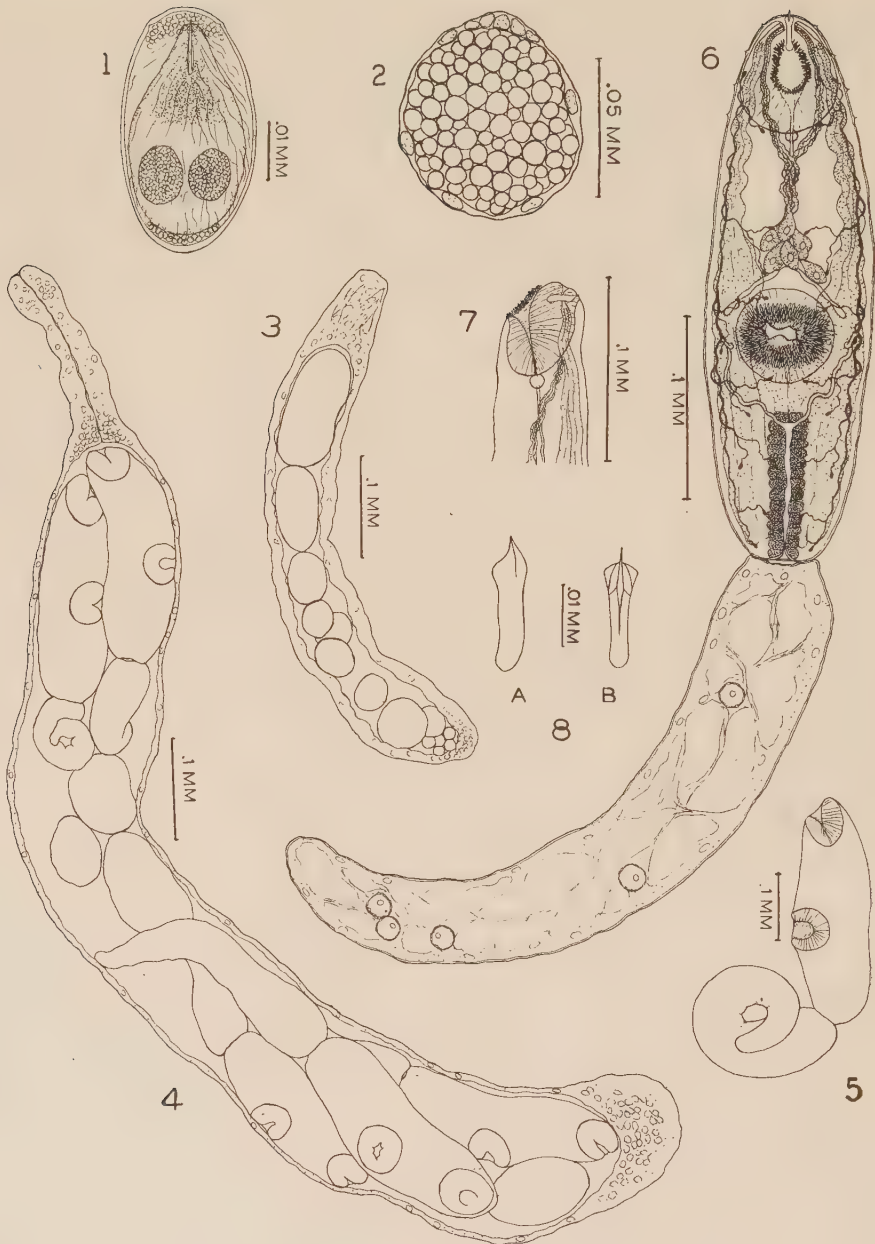
DESCRIPTION OF DEVELOPMENTAL STAGES

The egg and miracidium (Fig. 1).—Mature egg elongate oval in shape, dark brown in color, distinctly operculate, measuring $33\text{--}46\ \mu$ in length by $17\text{--}20\ \mu$ in breadth. Shell thick and uniform in structure except for slightly thickened posterior end. Fully developed miracidium faintly visible through opaque shell. Many refractile droplets present between shell and larva, particularly at anterior and posterior ends. Body of miracidium pear-shaped, with smaller anterior end directed toward operculum. Cilia of epidermis most numerous and longest at anterior end. Stylet slender, protruding slightly from anterior end and extending posteriorly about one-fourth of body length. Primitive gut opening through minute pore in anterior tip of body. Gut becoming broader and sac-like internally and filling most of anterior half of body. Two large oval oppositely situated vesicles containing highly refractile granules occupy posterior half of body. No epidermal plates, flame cells, or penetration glands detected in unhatched miracidia.

The mother sporocyst.—Mother sporocysts, observed on the 50th day of infection, were pearly white in contrast to the tan digestive gland of the host. A sporocyst developing on the surface of the gland appeared as an oval slightly raised papule, while others situated between the lobes of the gland were of various irregular shapes, their shape being determined by the space in which they were developing. Superficially, because of the bulging of the enclosed daughter embryos, the mother sporocyst has the appearance of a bunch of grapes. The thin delicate wall of the sporocyst fuses with the surrounding host tissue making it impossible to dissect it out whole. The matrix of the sporocyst is composed of irregularly shaped cells. The 50 to 70 daughter sporocysts developing in the meshes are dispersed rather uniformly through the matrix. All of the daughter embryos, in the same mother, are approximately in the same stage of development.

The immature daughter sporocysts.—The daughter embryos contained in the mothers on the 50th day were oval or slightly elongate structures (Fig. 2), capable of slight movement of the anterior end when freed in 0.5 per cent saline. They varied from 0.065 to 0.245 mm in length and from 0.057 to 0.126 mm in width. The wall of the smaller embryos consisted of a thin membrane composed of flattened cells, while the contents consisted of a mass of tightly packed separate germ cells. The larger embryos contained several clusters of germ cells, interpreted as being cercarial embryos at an early stage of development.

When next studied on the 64th day of infection, the daughter sporocysts, though still contained in the mothers, were at the migrating stage of development (Fig. 3).



EXPLANATION OF FIGURES

Drawings made with the aid of a camera lucida.

FIG. 1. Egg with mature miracidium, showing stylet and large refractive bodies.

FIG. 2. Very young embryo separated from mass of mother sporocyst 51 days after infection.

FIG. 3. Daughter sporocyst in the migrating stage 64 days after infection.

FIG. 4. Mature daughter sporocyst, with developing and mature cercariae.

FIG. 5. Lateral view of cercaria showing the ventral coiling of the tail.

FIG. 6. Ventral view of cercaria showing morphological details. The large and small penetration glands and their ducts are somewhat diagrammatic. Details of excretory system added freehand.

FIG. 7. Lateral view of anterior end of cercaria, showing position of stylet and openings of gland ducts.

FIG. 8. Stylet of cercaria. A—lateral view, B—dorso-ventral view.

They were elongated, cylindrical structures measuring from 0.25 to 0.60 mm in length and from 0.06 to 0.09 mm in diameter at the widest portion. The anterior end was attenuated into a solid "neck." The body cavity, which was fully formed, contained from 6 to 15 fairly large cercarial embryos and a group of smaller germinal elements. The group of small germ balls and cells, usually located at the posterior end of the cavity, is believed to be a germ mass from which additional cercarial embryos are formed. The largest cercarial embryos contained at this time measured 0.045–0.095 mm long by 0.025–0.042 mm wide. As yet, they showed no differentiation of cercarial characters.



FIG. 9. Adult. Ventral view, unflattened.

FIG. 10. Adult. Ventral view, showing details of excretory system.

The mature daughter sporocyst (Fig. 4).—Living sporocysts glistening white in appearance, slightly motile. Total length 0.8–1.2 mm. Sporocyst divided into anterior "neck" and body proper. "Neck" narrower than body, measuring 0.10–0.20 mm in length by 0.04–0.06 mm in diameter. Birth canal passing through center of "neck" visible only when cercariae are escaping or after preservation. Diameter of body 0.125–0.175 mm at widest portion. Body wall 3–5 μ thick and composed of a single layer of flattened cells except at posterior end where it is much thicker. Body cavity containing from 8 to 15 mature cercariae and several embryos of various sizes.

The cercaria (Figs. 5, 6, 7).—Body relatively transparent, elongate oval in shape and flattened slightly dorso-ventrally. Ten heat-killed specimens measure 0.285–

0.360 mm long by 0.066–0.087 mm wide in region of acetabulum. Tail large, round, tapering gradually from base to tip, and measuring 0.285–0.450 mm long by 0.066–0.108 mm wide near base. It is composed of five long radially arranged giant cells supported by numerous small cells. In living cercariae the content of the giant cells is fluid and the nuclei shift their positions readily. Cuticle aspinose and smooth except for an undetermined number of sensory papillae visible along lateral margins of body of living specimens. Oral sucker directed anteriorly, muscular, measuring 0.045–0.056 mm long by 0.042–0.045 mm wide, and bearing several irregular rows of fine spines around its subterminal opening. Stylet (Fig. 8) small, measuring 22.5–24 μ in length, 5–5.5 μ in width and 6.5–7 μ in depth, and lying in small pocket in dorsal wall of sucker. Acetabulum situated just posterior to middle of body, measuring 0.05–0.06 mm in diameter, and bearing several concentric rows of fine spines on its exposed surface. Pharynx globular, measuring 8.5–10 μ long by 9–13 μ wide. Esophagus long and slender, passing posteriorly to bifurcate into two short ceca just in front of acetabulum. Six pairs of large unicellular glands lying just beneath the lateral and dorsal surfaces occupy most of the posterior half of the body. The glands become attenuated and grouped into two bundles of six, one on each side of the body, anterior to acetabulum. Each group of glands passes through lateral walls of oral sucker and opens by a common duct into the stylet pocket. A group of six small penetration glands lies in midline just anterior to acetabulum. The ducts of these glands, after tangling together, separate into two groups of three ducts each. Each group of ducts passes laterally and anteriorly through oral sucker to stylet pocket just medial to large gland ducts. Excretory pore terminal in groove between body and tail. Excretory bladder simple, tubular, I-shaped, enclosed in large compact cells, and extending anteriorly almost to acetabulum to receive a common collecting tubule from each side of the body. Each common collecting tubule passes antero-laterally, then gives rise to an anterior and posterior main collecting tubule, each of which gives off three accessory tubules that branch into two capillaries. Each capillary tubule terminates in a flame cell establishing a flame cell formula of $2 [(2+2+2) + (2+2+2)]$. Germinal primordia visible in stained specimens as a compact group of deeply stained cells lying just posterior to acetabulum.

The metacercaria.—Three encysted metacercariae, believed to be those of *B. americanum* were obtained from the abdominal cavity of a cucumber beetle. The cysts were exceedingly delicate, and changes in osmotic relations caused them to rupture soon after they were dissected out in 0.5 per cent saline. In contrast to the yellowish-green beetle tissue, the cysts were a grayish white color. The outer portion of the cyst consisted of a fairly thick layer of host tissue while the inner portion consisted of a thin layer of transparent noncellular material. Except for the absence of a tail, the encysted cercariae were no different from nonencysted forms. They showed no increase in body size or development of the germinal primordia and still retained their stylets.

The adult worm (Figs. 9, 10).—Body thin, semi-transparent, elongated, with almost parallel sides, rounded anterior and tapering posterior ends, 2.11–5.67 mm long by 0.158–0.564 mm wide at acetabulum. Cuticle thin, aspinose, with small retractile sensory papillae, visible on margin of preacetabular region of body. Oral sucker weakly muscular, 0.10–0.34 mm in diameter, subterminal to a short lip-like

projection. Acetabulum weakly muscular, protrusile, 0.10–0.32 mm in diameter, situated within anterior fourth of body. Pharynx small, 0.030–0.080 mm in diameter. Esophagus narrow and very thin-walled, straight to slightly wavy, 0.103–0.386 mm long, bifurcating $\frac{1}{2}$ – $\frac{2}{3}$ the distance from oral sucker to acetabulum. Ceca of medium width, slightly wavy, passing lateral to genital organs, dorsal to vitellaria, and terminating just beyond the last vitelline follicles. Excretory pore terminal, excretory vesicle tubular, narrow, extending anteriorly to near anterior limits of vitellaria to receive a common collecting tubule from each side. Each of the common collecting tubules passes laterally and anteriorly to about posterior level of anterior testis to divide into an anterior and posterior main collecting tubule. Both of the main collecting tubules on each side of body give rise to three short accessory tubules, each of which branches into two capillaries. Each capillary tubule terminates in a single flame cell, thus establishing a 2 [(2+2+2) + (2+2+2)] type of flame cell pattern. Genital pore median at intestinal bifurcation. Testes large, distinctly lobed, approximately equal in size, 0.120–0.572 mm long by 0.090–0.400 mm wide. Anterior testis usually separated from acetabulum by a single uterine loop, posterior testis obliquely situated close behind anterior so that their zones partly overlap. Cirrus sac elongate, pyriform, 0.116–0.303 mm long by 0.050–0.103 mm wide, containing a much convoluted seminal vesicle and eversible cirrus. Ovary much smaller than testes, round to transversely oval, 0.070–0.255 mm in transverse diameter, situated to one side of body immediately behind posterior testis. Seminal receptacle small, globular, located dorsal to caudal margin of ovary. Mehlis' gland diffuse, situated medially just posterior to seminal receptacle. Laurer's canal not observed. Vitellaria each composed of 8–15 large oval follicles, located laterally just posterior to ovary. Uterus much convoluted, filling most of postovarial portion of body, then ascending by one of the three following courses to genital pore; 1, passing to either right or left of ovary, between ovary and posterior testis, between testes, then anteriorly by a wavy course; 2, passing to either right or left of both ovary and posterior testis, between testes, then anteriorly by a wavy course; 3, passing to either right or left of all three genital glands, then anteriorly by a wavy course. Mature ova few, dark brown, 33–46 μ long by 17–25 μ wide.

Hosts: *Cassidix mexicanus prosopidicola* (type), *Quiscalus versicolor*, *Sturnella magna*, *Corvus brachyrhynchos* and *Cyanocitta cristata*.

Habitat: Liver.

Localities: Eagle Lake, Tex. (type), Houston, Tex., Reelfoot Lake, Tenn., Athens, Ga., and Washington, Ga., U. S. A.

Specimens: U. S. Nat. Mus. Helm. Coll. Nos. 36752 (type), 36753 and 36754 (paratypes).

DISCUSSION

The genus *Brachylecithum* was established by Strom (1940) for a large group of closely related species formerly assigned to the genus *Lyperosomum* Looss, 1899. At present, the genus contains approximately 35 species and subspecies. As a group these are very delicate worms which show considerable morphological variation with different methods of handling. They also exhibit considerable variation as a result of host, habitat, age, and hereditary factors. Because of the variation shown by them, and because essential taxonomic characters are lacking for many

species which were inadequately described or described from poor material, it is impossible to determine with certainty whether or not *B. americanum* is a new species. However, since it is the first *Brachylecithum* to be reported from North American birds, it seems advisable to describe it as new. It differs from most species of the genus in having a relatively small ovary and large lobed testes situated so that their zones overlap. From the apparently related *B. papabejani* (Skrjabin and Udinzew, 1930), *B. americanum* differs in having smaller eggs and the acetabulum equal in size to the oral sucker rather than much smaller.

The morphology and development of the larval stages has been described for two species belonging to two genera of DICROCOELIINAE; *Dicrocoelium dendriticum* (Rudolphi, 1819) by Vogel (1929), Mattes (1933, 1936) and Neuhaus (1936, 1938) and *Eurytrema procyonis* Denton 1942, by Denton (1944). Of these two species, the larval forms of *D. dendriticum* show a closer affinity to those of *B. americanum* than do those of *E. procyonis*. The mother sporocysts are identical in structure and in the production of a single generation of daughter sporocysts, all of which mature simultaneously. The daughter sporocysts of both are elongated tubular structures with a distinct wall composed of a single layer of flattened cells. They both possess a birth canal. The daughter sporocysts of *D. dendriticum* are much larger than those of *B. americanum*.

The cercariae of the two species are similar with respect to general shape of the body and tail; a small stylet is present in both, large and small penetration glands occupy similar positions, and the excretory systems are identical in pattern. The cercaria of *D. dendriticum* is approximately twice the size of that of *B. americanum* and has twice as many large posterior penetration glands. The number of small penetration glands is the same for both species.

The cercariae of both *D. dendriticum* and *B. americanum* are deposited on vegetation in the same manner by the snail hosts. The cercariae of *D. dendriticum*, however, form group cysts on vegetation and are infective directly for the final hosts, while, according to the evidence here presented, those of *B. americanum* may utilize an encystment host.

SUMMARY

Ova of *Brachylecithum americanum* n. sp., when fed to *Polygyra texasiana* (Moricand) and *Praticollega berlandieriana* (Moricand), develop into irregularly-shaped mature mother sporocysts in 64 days. Within the matrix of the mother sporocysts a single generation of 50 to 70 daughter sporocysts develops simultaneously. The daughter sporocysts escape by rupture of the mother between the 64th and 70th day of infection and migrate to the walls of the mantle cavity and adjoining tissues to continue their development. Cercariae, mature after the 105th day of infection, escape through the birth canal of the sporocysts and collect in the mantle cavity of the host. Masses of 150 to 300 cercariae are expelled from the respiratory pore and are deposited on vegetation by the snails. The cercariae possess a long tail, a stylet, both large and small penetration glands, and a flame cell formula of $2 [(2+2+2) + (2+2+2)]$. Evidence is presented that the cercariae enter larvae of chrysomelid beetles which then serve as second intermediate hosts, and as a means of transfer to the definitive host. The adult worm is a common parasite of birds of the families CORVIDAE AND ICTERIDAE.

The larval stages of *B. americanum* are compared with those of *Dicrocoelium dendriticum* (Rudolphi, 1819), which they resemble very closely.

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AN ECOLOGICAL STUDY OF THE HELMINTH PARASITES OF AMPHIBIANS AND REPTILES OF WESTERN MASSA- CHUSETTS AND VICINITY

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Comparatively few species of helminth parasites have been described from New England fresh-water and terrestrial vertebrates. Practically nothing has been published concerning the ecology of such parasites. While associated with the Biology Department of Amherst College, Amherst, Mass., from 1936 to 1941, the writer carried on a survey of helminths of the more common species of vertebrates found in that region. Records were made of physical conditions of localities in which hosts were collected, position of worm in the host, condition of host, season, life cycles of parasites (when possible), etc., as well as the morphology and taxonomy of parasites found.

Elucidation of several trematode life cycles resulted from this study (Rankin, 1939, 1939a, 1944, 1944a), in which type of life cycle was correlated with as many ecological factors as possible. The present paper summarizes and discusses the data obtained from examinations of amphibians and reptiles. Those for birds and mammals will appear in a later publication. A total of 262 hosts was examined, including 13 species of amphibians and six species of reptiles. From these animals, 27 species of helminth parasites were collected: 12 trematodes, 10 nematodes, 4 cestodes, and 1 acanthocephalan.

It is a pleasure to acknowledge the assistance of former colleagues at Amherst College, particularly of Dr. O. E. Schotté and Mr. Carl Holthausen, in the collection and care of animals studied.

MATERIALS AND METHODS

Animals were placed in cloth sacks immediately upon collection with damp moss or leaves to prevent desiccation. By this procedure they could be kept alive in a cold room (c. 4° C) for two or three weeks. At the laboratory, the smaller animals were dissected directly under the dissecting microscope, whereas the larger ones were eviscerated first and then the body contents studied under the microscope. All helminths were placed in Amphibian Ringer's Solution as they were encountered and studied alive at the end of each autopsy.

Trematodes were killed by placing them on a slide with a drop of water, covering with cover-glass, placing F.A.A. fixative at one edge, and drawing it under the cover-glass by means of filter paper placed at the opposite edge. Uniform fixation without distortion was insured by this method. Whole mounts were made from specimens stained in Borax-Carmine, Lynch's precipitated method, and mounted in balsam.

Cestodes were stretched around a glass plate and killed by painting them with F.A.A. fixative. Whole mounts were made from scolices and representative proglottids stained in dilute Delafield's hematoxylin and mounted in balsam.

Nematodes and acanthocephalans were killed by plunging them into hot 70%

SUMMARY OF HOSTS AND PARASITES ENCOUNTERED

Number of hosts examined, parasites found, and total per cent infection of each species of host with the species of parasite, are summarized as follows:

Host	No. ex- amined	Parasite	Per cent in- fected
AMPHIBIA: CAUDATA			
<i>Ambystoma jeffersonianum</i> (Green)	1	<i>Brachycoelium salamandrae</i>	100
<i>Ambystoma maculatum</i> (Shaw) ..	13	<i>B. salamandrae</i>	84 +
<i>Desmognathus f. fuscus</i> (Raf.) ...	13	<i>B. salamandrae</i>	15 +
		<i>Oswaldocruzia pipiens</i>	23 +
<i>Eurycea b. bislineata</i> (Green)	9	<i>B. salamandrae</i>	100
		Spirurid cysts	11 +
<i>Plethodon cinereus</i> (Green)	35	<i>B. salamandrae</i>	25 +
		<i>Cosmoceroides dukae</i>	8 +
		<i>O. pipiens</i>	3
<i>Triturus v. viridescens</i> Raf.	138	<i>Plagiotura parva</i>	5 +
		<i>P. salamandra</i>	42 +
		<i>B. salamandrae</i>	15 +
		<i>Gorgoderina attenuata</i>	29 +
		<i>Megalodiscus temperatus</i>	16 +
		Spirurid cysts	50 +
		<i>Capillaria tenua</i>	35 +
		<i>Camallanus</i> sp., larva	0.08 +
		<i>Physaloptera</i> sp., immature	0.08 +
		<i>C. dukae</i>	3 +
		<i>Bothriocephalus rarus</i>	0.08 +
		<i>Pomphorhynchus bulbocolli</i>	0.08 +
		<i>Plerocercoid larvae</i>	0.08 +
AMPHIBIA: SALIENTIA			
<i>Hyla crucifer</i> Wied.	6	<i>O. pipiens</i>	50
		<i>Glyptelminis quieta</i>	18 +
		<i>C. dukae</i>	18 +
		<i>Rhabdias ranae</i>	18 +
<i>Rana catesbeiana</i> Shaw	1	<i>G. attenuata</i>	100
		<i>Cephalogonimus americanus</i>	100
		<i>Halipegus amherstensis</i>	100
		<i>Cylindrotaenia americana</i>	100
		<i>C. dukae</i>	100
<i>Rana clamitans</i> Latreille	10	<i>G. attenuata</i>	60
		<i>G. quieta</i>	30
		<i>Loxogenes bicolor</i>	10
		<i>Haematoloechus medioplexus</i>	20
		Spirurid cysts	20
		<i>C. dukae</i>	40
		<i>Capillaria tenua</i>	20
		<i>O. pipiens</i>	60
<i>Rana pipiens</i> Schreber	3	<i>G. attenuata</i>	33 +
		<i>H. medioplexus</i>	33 +
<i>Rana palustris</i> LeConte	9	<i>G. attenuata</i>	22 +
		Gorgoderid cysts	22 +
		<i>H. medioplexus</i>	11 +
		<i>O. pipiens</i>	55
		<i>B. salamandrae</i>	11 +
		<i>R. ranae</i>	22 +
		<i>C. tenua</i>	11 +
<i>Rana sylvatica</i> LeConte	11	Gorgoderid cysts	27 +
		Cestode cysts	9 +
		<i>O. pipiens</i>	100
		<i>R. ranae</i>	45 +
		<i>Plerocercoid larvae</i>	9 +
<i>Bufo fowleri</i> Hinckley	1	<i>O. pipiens</i>	100
REPTILIA: TESTUDINATA			
<i>Clemmys insculpta</i> (LeConte)	1	Negative	0
REPTILIA: SERPENTES			
<i>Coluber c. constrictor</i> L.	1	<i>Kalicephalus a. flagellus</i>	100
		<i>Polydelphis</i> sp.	100
		<i>Capillaria</i> sp.	100
<i>Lampropeltis t. triangulum</i> (Lace- pede)	1	Cestode cysts	100
		<i>R. ranae</i>	100
		<i>Capillaria</i> sp.	100
<i>Storeria dekayi</i> (Holbrook)	2	Negative	0
<i>Storeria occipito-maculata</i> (Storer)	1	<i>C. dukae</i>	100
<i>Thamnophis s. sirtalis</i> (Lin.)	6	<i>G. attenuata</i>	16 +
		Strigeid metacercariae	33 +
		<i>Zeugorchis aequatus</i>	16 +
		Cestode cysts	16 +
		<i>K. a. flagellus</i>	16 +
		<i>R. ranae</i>	33 +

alcohol. They were then transferred to a mixture of 70% alcohol and a few drops of glycerine. Some were mounted in balsam after gradual infiltration with Borax-Carmine stain. A few anterior ends of nematodes were mounted in glycerine jelly to present an *en face* view.

The lack of balance between the numbers of different species of hosts examined is chiefly a reflection of their relative abundance. The newt (*Triturus*) is by far the most common cold-blooded vertebrate in the aquatic habitats studied. The red-backed salamander (*Plethodon cinereus*) is very abundant in certain terrestrial areas, rare in others. The various species of frogs are about equally represented, although the record for the bullfrog (*Rana catesbeiana*) does not seem to show this. Ease of collecting is a pertinent factor. Salamanders are fairly easy to catch whereas frogs prove more difficult. The number of examinations for reptiles is regrettably low. These animals occur sporadically, except for the common striped snake (*Thamnophis s. sirtalis*).

ECOLOGY OF PARASITES ENCOUNTERED

Trematoda

1. *Brachycoelium salamandrae* (Froehlich), Dujardin, 1845.

Hosts: *Ambystoma jeffersonianum*, *A. maculatum*, *Desmognathus f. fuscus*, *Eurycea b. bislineata*, *Plethodon cinereus*, *Triturus v. viridescens*, *Rana palustris*.

Position: Intestine.

The synonymy and relationships of this trematode have been reviewed by Rankin (1938). Worldwide in distribution, *B. salamandrae* is one of the commonest amphibian trematodes encountered (Brandt, 1936; Rankin, 1937), occurring in 53% of the amphibians studied. A correlation seems to exist between occurrence of this fluke and habitat of the host. In the strictly aquatic salamander, *Triturus*, only 15% infection is found. Likewise, but few worms per host are observed. On the other hand, terrestrial salamanders are heavily infected (25–100%). Whenever large numbers of flukes are present, the majority are quite small. Conversely, when but few (below 20) are present, individuals are usually much larger in size.

2. *Plagitura parva* Stunkard, 1933, and *P. salamandra* Holl, 1928.

Host: *Triturus v. viridescens*.

Position: Intestine.

Stunkard (1936) considers the two species of *Plagitura* to be specifically valid, largely on size differences at maturity. In the present study, such great variation is found in size and maturity that much doubt is thrown on the validity of these two species. As in infection with *Brachycoelium*, heavy infections tend to produce many small flukes, whereas light infections are usually made up of large specimens. The same discrepancy was found earlier (Rankin, 1937), so much so, that in collecting all specimens were lumped together in one species. Studies on other genera of trematodes indicate that host species play an important role in determining adult morphological variations in flukes (Rankin, 1938, 1944, 1944a).

This trematode is found in only one species of host in the aquatic habitat.

3. *Gorgoderina attenuata* Stafford, 1902.

Hosts: *Triturus v. viridescens*, *Rana catesbeiana*, *R. clamitans*, *R. pipiens*, *R. palustris*, *R. sylvatica*, *Thamnophis s. sirtalis*.

Position: Bladder.

The life cycle of this fluke, with a concomitant review of the literature was made by Rankin (1939). It was found that cystocercous cercariae, liberated into the water, were ingested by tadpoles, in which the young flukes penetrated to the coelom and encysted. The worms become adult in the bladder of the definitive host when the latter eat the tadpoles. The occurrence of metacercariae in the coelom of adult hosts, therefore, may be accounted for by the penetration of cercariae during the tadpole stage of the host. Likewise, such a life cycle determines the occurrence of this worm in aquatic hosts. Such hosts are usually susceptible to parasitism with this fluke.

4. *Glyptelminis quieta* (Stafford, 1900) Stafford, 1905.

Hosts: *Hyla crucifer*, *Rana clamitans*.

Position: Intestine.

This parasite occurs only in aquatic hosts. Rankin (1944) showed that the metacercariae develop in the epithelium of frogs and that the worms become adult when frogs eat sloughed epithelium. The two localities in which this parasite was found, are very small, transient ponds.

5. *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932.

Host: *Triturus v. viridescens*.

Position: Bladder.

The genus *Megalodiscus* has been well reviewed by Bravo (1941) and the life cycle of *M. temperatus* by Krull and Price (1932). The occurrence of this fluke is limited primarily to two permanent ponds in Pelham, each connected by a small canal.

6. *Cephalogonimus americanus* Stafford, 1902.

Host: *Rana catesbeiana*.

Position: Intestine.

It is believed that this is an accidental infection. The locality from which the single host was collected is a very small sink-hole abounding in muskrats heavily infected with this fluke. Brandt (1936), however, has recorded it from anuran hosts.

7. *Halipegus amherstensis* Rankin, 1944.

Host: *Rana catesbeiana*.

Position: Mouth cavity and Eustachian tubes.

The life cycle and relationships of this fluke have been reviewed by Rankin (1944a). Metacercariae develop in copepods.

8. *Loxogenes bicolor* Krull, 1933.

Host: *Rana clamitans*.

Position: Cysts in bile duct.

A single case of infection was obtained with this fluke.

9. *Haematoloechus medioplexus* Stafford, 1902.

Hosts: *Rana clamitans*, *R. pipiens*, *R. palustris*.

Position: Lungs.

Krull (1931) showed that metacercariae of this species develop in aquatic insect larvae. Members of this genus are common parasites of frogs (Brandt, 1936; Harwood, 1932).

10. *Zeugorchis aequatus* Stafford, 1905.

Host: *Thamnophis s. sirtalis*.

Position: Stomach.

A single infection was encountered.

11. Strigeid metacercariae.

Host: *Thamnophis s. sirtalis*.

Position: Cysts on mesentery.

A single infection of this larva was found. Usually adult in fish-eating birds and mammals, the presence of this larva in a reptile is probably accidental.

Cestoda

1. *Bothriocephalus rarus* Thomas, 1937.Host: *Triturus v. viridescens*.

Position: Intestine.

Only one infection with this tapeworm was found.

2. *Cylindrotaenia americana* Jewell, 1916.Host: *Rana catesbeiana*.

Position: Intestine.

A single infection was found.

3. Plerocercoid larvae.

Hosts: *Rana sylvatica*, *Triturus v. viridescens*.

Position: Intestine.

Single infections were obtained in each species of host.

4. Cestode cysts.

Host: *Thamnophis s. sirtalis*.

Position: Mesenteries and heart.

A single infection was found.

Nematoda

1. *Oswaldocruzia pipiens* Walton, 1929.Hosts: *Desmognathus f. fuscus*, *Plethodon cinereus*, *Hyla crucifer*, *Rana clamitans*, *R. palustris*, *R. sylvatica*, *Bufo fowleri*.

Position: Foregut.

A widely distributed nematode (Harwood, 1932), this parasite is commonly found in aquatic hosts, rarely in terrestrial. It is the commonest nematode found in this study.

2. *Cosmocercoides dukae* (Holl, 1928) Travassos, 1931.Hosts: *Plethodon cinereus*, *Triturus v. viridescens*, *Hyla crucifer*, *Rana catesbeiana*, *R. clamitans*, *Storeria occipito-maculata*.

Position: Hindgut.

Harwood (1932) found this nematode to be one of the most widely distributed of all encountered. It ranges in both aquatic and terrestrial habitats.

3. *Capillaria tenua* Mueller, 1932.Hosts: *Triturus v. viridescens*, *Rana clamitans*, *R. palustris*.

Position: Lining of walls of foregut.

Predominant in *Triturus*, it is occasionally found in the other two hosts.4. *Capillaria* sp.Hosts: *Coluber c. constrictor*, *Lampropeltis t. triangulum*.

Position: Foregut.

Although immature, the species is very similar to *C. serpentina* Harwood, 1932.

5. *Rhabdias ranae* Walton, 1929.Hosts: *Hyla crucifer*, *Rana palustris*, *R. sylvatica*, *Lampropeltis t. triangulum*,

Thamnophis s. sirtalis.

Position: Lungs.

Few specimens were found in each of the hosts.

6. *Kalicephalus agkistrodontis flagellus* Harwood, 1932.

Hosts: *Coluber c. constrictor*, *Thamnophis s. sirtalis*.

Position: Intestine.

Few specimens were found in each of the hosts.

7. *Polydelphis* sp.

Host: *Coluber c. constrictor*.

Position: Intestine.

Only one infection was found.

8. *Camallanus* sp.

Host: *Triturus v. viridescens*.

Position: Intestine.

Only one immature specimen was found.

9. *Physaloptera* sp.

Host: *Triturus v. viridescens*.

Position: Intestine.

Only one immature specimen was found.

10. Spirurid cysts.

Hosts: *Eurycea b. bislineata*, *Triturus v. viridescens*, *Rana clamitans*.

Position: Wall of foregut and stomach.

Over 50% of the newts are infected with this larval nematode. It is clearly correlated with the aquatic habitat.

Acanthocephala

1. *Pomphorhynchus bulbocolli* Linkins, 1919.

Host: *Triturus v. viridescens*.

Position: Midgut.

A common parasite of the sucker (*Catostomus c. commersonnii*), this worm is probably accidental in the single amphibian host.

DISCUSSION AND CONCLUSIONS

Correlation between type of habitat and species of parasite has been observed by many investigators (Pearse, 1924, 1926; Ingles, 1936; Rankin, 1937; etc.). Some species of trematodes are limited to hosts of a fairly narrow range of habitat distribution. *Brachycoelium salamandrae* is limited primarily to hosts in terrestrial habitats. Aquatic hosts might become infected either during a terrestrial larval stage (*Triturus*) or during occasional migrations to land (*Desmognathus*). *B. salamandrae* was the only trematode found in land turtles by Rumbold (1928). Its prevalence in terrestrial hosts was observed by Rankin (1937). Although the life cycle of this fluke is not known as yet, from a study of hosts' stomach contents as well as of hosts' distribution, it would seem that terrestrial invertebrates are involved. *Plagitura* spp. are limited to hosts in aquatic habitats. Stunkard (1936) described the life cycle of *P. parva*. He showed that metacercariae develop in fresh-water snails and insect larvae. The only chance for other amphibian hosts to become infected would be during the breeding season when such animals return to water. This would account for its rare occurrence in hosts other than *Triturus* (Rankin, 1937).

Along with *Plagitura*, the following trematodes seem to be specific for the aquatic habitat: *Gorgoderina attenuata*, *Glypthelmins quieta*, *Megalodiscus temperatus*, *Cephalogonimus americanus*, *Halipegus amherstensis*, *Loxogenes bicolor*, and *Haematoloechus medioplexus*. Little (1928) found aquatic more heavily infected than land salamanders, as did Rankin (1937). Rumbold (1928) and Brandt (1936), studying turtles and salientians respectively, came to this same general conclusion.

The occurrence of tapeworms is essentially correlated with an aquatic environment. Copepods are known to serve as intermediate hosts for salamander cestodes (Thomas, 1931). These crustaceans abound in the various aquatic areas studied.

Nematodes are distributed fairly evenly throughout the habitats studied, although there is a tendency for them to be found in few host species. *Oswaldocruzia pipiens*, *Capillaria tenua*, and spirurid cysts tend to be associated with the aquatic habitat. Acanthocephala are generally associated with a host in all stages of their development (Van Cleave, 1919). *Pomphorhynchus bulbocolli*, usually found in fishes, is probably limited to the aquatic region through its life cycle.

Locality within the host and the parasite's life cycle usually determine the habitat in which the parasite is found. For example, *Glypthelmins quieta* and *Gorgoderina attenuata* develop metacercariae in aquatic intermediate hosts and are limited, therefore, to aquatic definitive hosts; etc. On the other hand, host-specificity may determine a parasite's distribution. *Megalodiscus temperatus* and *Plagitura* sp., with aquatic intermediate hosts, are found in but one species of definitive host, *Triturus*. *Gorgoderina attenuata* tends to be limited to species of *Rana*. *Cephalogonimus americanus*, *Halipegus amherstensis*, *Loxogenes bicolor*, and the cestodes are found in single host species. This indicates that some species of helminths may be limited physiologically to single hosts. Although the data indicate that many of the observed parasites are specific for single hosts, elucidation of the life cycle may show that specificity, as such, is not the only factor determining distribution. Many species of helminths are, however, found in many species of hosts. *B. salamandrae* is found in seven different host species, *G. attenuata* in seven, *O. pipiens* in seven, and *C. dukae* in six. By far the majority of species are found in amphibian hosts. Habitat, life cycle, and host-specificity are, therefore, closely interrelated. A knowledge of all factors involved is necessary to determine the limiting one.

Age and size of, and seasonal variation within, a habitat may influence the extent of parasitism present. Small, old and permanent areas with no outflow tend to build up an infection once started. Such conditions may be more conducive to maintaining a high degree of parasitism than a larger, more fluid habitat with constant change within it. In Whately Glen Pond, *Triturus* is infected with seven species of helminths; in Leverett Pond with eight; in Ive's Ice Pond with five. On the other hand, the terrestrial habitats show hosts with few species of parasites. Likewise, accidental and multiple infections should be more common in small permanent habitats. *Triturus* is commonly found infected with six or more species of helminths, 13 different species collected in this study. *Rana* spp. usually harbor five or more species of flukes. Conversely, in the terrestrial habitat, *Ambystoma* sp. yielded but one species of helminth; *Plethodon*, one or two. Accidental infection with *Pomphorhynchus* and *Cephalogonimus* is found in small isolated ponds.

When large infections of a single species are found, the worms are usually small,

though mature. When few worms are present, however, they tend to be correspondingly much larger. Crowding may account for this size variation (Rankin, 1937). The fewer the parasites, the more room and food for each individual. The validity of specific identification may be influenced by this condition, particularly with respect to *Plagitura parva* and *P. salamandra*. Stunkard (1936) believes these two trematodes to be distinct species. Difficulty has been experienced in the present study in defining these two species. It is believed that this controversy warrants a brief consideration. The writer (Rankin, 1938, 1944, 1944a) has found that many so-called specifically valid characters are too variable to be of taxonomic value. Other investigators have come to this same general conclusion (Chandler, 1923; Venard, 1938). In hermaphroditic animals, with little or no chance for cross-fertilization, there may be built up over a period of time an isogenic stock, in which succeeding generations would be morphologically alike. If individuals of various phenotypes are obtained, then the differences should be environmental, not genetic. For example, if the metacercariae resulting from a single miracidium of an isogenic stock are fed to several species of definitive hosts, and many variations are obtained in the adult worms, these phenotypic expressions are probably due to the varied environments. The many recognized species of a given genus, therefore, may be but environmental variations of the same species.

As far as can be determined the distribution data recorded here are new.

SUMMARY

A taxonomic and ecological survey has been made of helminth parasites of amphibians and reptiles in western Massachusetts and vicinity. Two hundred and sixty-two hosts were examined. Twenty-seven species of helminths were collected: 12 trematodes, 10 nematodes, 4 cestodes and 1 acanthocephalan. Correlation of the data on the helminths with that on the habitats of the hosts was attempted. Aquatic habitats seem to lead to infection with more species and numbers of parasites, more chance of accidental and multiple infections, than do terrestrial habitats. Distribution of helminths depends on many factors, including habitat, life cycle of host and parasite, and host-specificity. Such variation is found in taxonomic characters of some flukes, as in *Plagitura* species, that doubt is raised as to their validity. A possible explanation of such conditions is offered in that many variations in an isogenic stock may be due to environmental rather than genetic factors. The distribution records for the helminths found are new.

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RESEARCH NOTES

A NEW LOCALITY FOR *TRYPANOSOMA CRUZI* IN ARIZONA

Kofoed and Whitaker (1936, *J. Parasitol.* **22**: 259-263) reported Reduviid bugs infected with *Trypanosoma cruzi* from Arizona (Tucson) and added another species of insect vector, *Triatoma uhleri*, to the list of those harboring *T. cruzi* in the United States; 7 of 79 specimens of *Triatoma uhleri* were infected, whereas the *Triatoma protracta* found at Tucson were uninfected. Wood (1942, *Am. J. Trop. Med.* **23**(3): 315-320) who did extensive collecting in 1939, 1940, and 1941 in the vicinity of the Alvarado Mine in Arizona, found 28, or 4.91%, of 570 bugs examined infected with *T. cruzi*. Among the infected bugs were *Triatoma longipes* and *T. rubida*. *T. protracta*, *Paratriatoma hirsuta*, and *T. protracta woodi* (?) were also collected but were uninfected.

During the period from June to November, 1943, due to the courtesy of Mr. W. J. Cummings, the author received 6 shipments of Reduviid bugs from the Tres de Mayo Mine, 15 miles north-east of Nogales, Arizona. Nogales is a hitherto unreported locality for *T. cruzi*, although in 1941 3 out of 7 bugs from this vicinity shipped to Dr. Dorland J. Davis, U. S. Public Health Service, were found infected with *T. cruzi*. Shipments made to Dr. Davis in 1942 contained no infected individuals.

The first shipment to the author in June, 1943, included 1 small, uninfected nymph and 20 dead *Triatoma longipes* which were too dried out to maintain an infection. In the second shipment (July) active crithidia and metacyclic forms of *Trypanosoma cruzi* were found in 1 out of the 3 *Triatoma longipes* sent. The infected individual was a large nymph which was teeming with the flagellates. The next shipment (August) included 2 live and 4 dead *T. longipes*. Of the 4 dead Reduviids, 2 were only recently dead and were found to be heavily infected. One of the two living bugs was also teeming with crithidial and metacyclic forms. In the fourth shipment (September), of 5 nymphs and 2 adult *T. longipes*, 2 nymphs and 1 adult bug were found infected. The next shipment (October) included 2 *Triatoma rubida*, one of which was infected; and in the next shipment (November) 1 out of the 2 *T. longipes* was infected. Thus of 16 *T. longipes* examined, 8, or 50%, were found infected; and of 2 *T. rubida*, 1, or 50%, was infected.

Since only a few bugs were examined, however, this high rate of infection may hold no statistical significance, although it is interesting to note that 3 out of 7 bugs (42.8%) sent to Dr. Davis from this locality in 1941 were infected.

All the specimens mentioned in this paper were collected in and around the house of Mr. W. J. Cummings. His house is a large adobe structure with white stucco walls to which many insects are attracted by lights within, or by reflected moonlight. Two hundred yards south of the camp is located the inner portal of a mine tunnel which is supported by a heavy loose-rock wall. In it is a group of wood-rat nests which have been occupied, according to Mr. Cummings, for the past 12 years. Due to this steady occupancy, Mr. Cummings thinks it probable that this is the site of the mammalian host of the *Trypanosoma cruzi* and the source of the infection. Other wood-rat nests in the vicinity are sparsely scattered among heavy rocks and cannot be opened for examination.—BETTY R. SCHUCK, *Department of Zoology, University of California*.

INTESTINAL MYIASIS WITH *LUCILIA*

Infections with this genus of blowflies seem rare enough to warrant recording.

The patient, an Italian Prisoner of War aged 21, gave a history of intermittent diarrhea for six or seven months, with the passage of, "small worms," recently—several on the morning of December 1, 1943. A single living third-stage larva of *Lucilia* sp. was passed on December 2. Hexylresorcinol was given that day, after which several adult *Ascaris lumbricoides* were recovered, but no more maggots.—J. DAN WEBSTER, *Second Lieutenant, Sanitary Corps, A.U.S., Utah ASF Depot, Ogden, Utah*.

THE SYRIAN HAMSTER, *CRICETUS AURATUS*, HOST OF *HYMENOLEPIS NANA*

The golden hamster, *C. auratus*, is a favorable host for many species of animal parasites and is being used increasingly in American laboratories as an experimental animal. Fecal examination of a shipment purchased recently from a commercial supply company, disclosed that several of the animals were infected with *Hymenolepis nana*. Since the cestode is a common parasite of rats and mice, its presence in the hamster is not surprising. But the worm is also a parasite of man, the eggs are numerous and very small and the contained oncospheres may develop in either the definitive or in an intermediate host. If as Chandler believes, "human infection is commonly acquired from eggs derived from rodents," laboratory workers and animal house attendants should be aware of possible contaminative infection.—HORACE W. STUNKARD, *New York University, University Heights, New York*.

AMERICAN SOCIETY OF PARASITOLOGISTS

THIRTY-FOURTH COUNCIL MEETING, BALTIMORE, MARYLAND

DECEMBER 16, 1944

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. H. E. Ewing, President of the Society, at 2:00 PM, December 16, 1944, in the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland. Past-president W. W. Cort, Treasurer-elect R. M. Stabler, and the following officers and councillors were present: J. T. Culbertson, H. E. Ewing, G. F. Otto, E. W. Price, B. Schwartz, H. W. Stunkard, and W. H. Wright.

I. REPORTS OF OFFICERS

1. *Secretary (J. T. Culbertson)*: As of December 15, 1944, there were 570 persons on the membership roll of the Society of whom 508 lived within and 62 lived outside the continental United States. Of the total number on the roll, 464 were members in good standing and 106 were delinquent for one (50), two (31), or three (25) years. Of those persons in good standing, 423 lived within and 41 lived outside the continental United States. Sixty-six new members were elected during the year, of whom 61 were domestic and 5 foreign.

The death of one member occurred since the last Council meeting: Dr. H. L. Van Volkenberg, Professor of Parasitology, Agricultural and Mechanical College of Texas, College Station, Texas, died on October 13, 1944.

The secretary's report was accepted as read, and placed on file.

2. *Treasurer (G. F. Otto)*: The Treasurer's report was read by G. F. Otto. A summary of this report, prepared by Dr. Otto for publication, follows:

TREASURER'S REPORT FOR THE FINAL YEAR 1944 (Dec. 6, 1943 to Dec. 1, 1944)

Receipts

Balance on hand Dec. 6, 1943		\$2957.16
Collected during current year:		
348 Member dues applying 1944	\$1383.50	
17 Member dues applying 1945	74.50	
2½ Member dues applying after 1945	10.37	
31 Member dues arrearages	123.50	
1 Member contribution	10.00	
		\$1601.87
3 Subscriptions to Vol. 29	13.50	
432 Subscriptions to Vol. 30	1965.43	
148 Subscriptions to Vol. 31	673.80	
7 Subscriptions beyond Vol. 31	32.00	
		2684.73
Back volumes, numbers & portraits	449.06	
Advertisements	119.92	
Author's charges	85.80	
Twenty-five volume index	53.00	
Interest on savings account	12.52	
Miscellaneous	15.30	
		735.60
		5022.20
		\$7979.36

Debits

Printing and handling Journal	
Volume 29	\$ 598.53
Volume 30	2372.63
	\$2971.16

Expenses in office of Chr. Ed. Comm.	143.16	
Expenses in office of Secretary	253.12	
Expenses in office of Treasurer	493.66	
		889.94
Miscellaneous	33.05	
		\$3894.15
Balance on hand, Dec. 1, 1944		4085.21
		\$7979.36

Respectfully Submitted
and Certified Correct
signed G. F. OTTO, *Treas.*

Audited and found correct
ROBERT M. STABLER, *Chr.*
W. W. CORT

Dr. Otto suggested that, since the Society has been operating at a profit, use of surplus should be considered, possibly for expanding the Journal or for establishing an endowment fund to pay dues of older members. Dr. Stunkard advised that surplus be kept liquid to provide for post-war emergencies. The Treasurer's report was accepted, subject to audit, and placed on file.

Dr. Otto moved that Council authorize the Secretary to turn the funds of the Society over to the Treasurer-elect, R. M. Stabler. Motion carried and the necessary documents were properly executed.

II. REPORTS OF COMMITTEES

1. *Custodian of Princeton Secretarial Fund (N. R. Stoll)*: No report has been received since that submitted in September, 1944, and published in minutes of the Thirty-Third Council Meeting.

2. *Chairman of the Editorial Committee (H. W. Stunkard)*: The Journal itself represents the chief part of the Editorial Committee's report. In 1944, the Journal will consist of almost 400 pages made up of 83 manuscripts. At present, articles appear approximately four months after being accepted for publication. An August supplement to the Journal was published which included titles and abstracts of 40 papers submitted for the general program in Cleveland as well as 7 titles and 4 abstracts for a symposium on "Parasitology in Relation to the War." Although the general program had to be cancelled almost at the last moment, the symposium was held.

3. *Auditing Committee (R. M. Stabler, Chairman, and W. W. Cort)*: The Auditing Committee approved the annual report of the Treasurer to December 1, 1944, and the report of the Custodian of the Princeton Secretarial Fund to September 7, 1944. Upon motion, the Committee's report was accepted and placed on file.

III. REPORTS OF REPRESENTATIVES

1. *Representatives to Council of A.A.A.S.*: No report was received from the representatives to the Council of A.A.A.S.

2. *Representatives to Council of U.A.B.S.*: No report was received since that submitted at the Cleveland meeting.

IV. NEW BUSINESS

1. *Election of New Members*: Council elected to membership in the Society Major Hildrus A. Poindexter, M.C., A.U.S., on leave from Howard University, Medical School, Washington, D.C.

2. *Management of Journal*: Dr. Stunkard pointed out that but few Manuscripts were submitted for publication in the past year but expected a considerably larger number soon after the war's end. He mentioned that certain agencies had urged the journal to include reviews on foreign papers, especially those in Latin American journals, but that no action had yet been taken since the publication of such reviews was contrary to Journal policy.

Dr. Otto moved that By-Law of the Society under "Management of the Journal of Parasitology" (No. 2) be changed to read: "The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. These shall be elected on the basis of attainment, interest in the Society, geographical location, and representation of the various fields of the science." The motion was seconded and carried.

3. *Place for the Meeting in 1945*: It was moved that the place for a general meeting in 1945 not be selected at this time. Motion carried.

4. *Appointments*: President H. E. Ewing appointed the following persons to be representatives of the Society or members of Society committees:

a. Committee on nomenclature: E. W. Price (Chairman), B. G. Chitwood, and A. McIntosh. (Note below appointment to Committee on nomenclature of N. R. Stoll, ex officio.)

b. Committee on common names of parasites: P. D. Harwood (Chairman) and D. B. McMullen.

c. Committee on terminology of strains of avian malaria: C. G. Huff (Chairman), G. H. Boyd, and R. D. Manwell.

d. Representatives to Council of Union of American Biological Societies: A. O. Foster and A. C. Walton.

e. Representatives to Council of American Association for the Advancement of Science: J. E. Ackert, and H. J. Van Cleave.

Dr. Wright moved that Dr. N. R. Stoll be appointed an ex-officio member of the Committee on Nomenclature, in view of his membership on the International Commission of Zoological Nomenclature. Motion carried.

It was moved and seconded that the President appoint a Committee to select a new Secretary for 1945, if the present incumbent found it impossible to continue in office. Motion carried.

The meeting was adjourned at 4:50 PM.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary*.